FORM PTO-1390 (REV 10-2000)

U.S DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

ATTORNEY'S DOCKET NUMBER: 205,209

U.S. APPLICATION NO. (If known, see 37 CFR 1.5): 09/868608

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/UA98/000020	December 16, 1998	

TITLE OF INVENTION: METHOD FOR TREATMENT OF PATIENTS USING EMBRYONIC CELL SUSPENSIONS

APPLICANT(S) FOR DO/EO/US: Alexandr Ivanovich SMIKODUB

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. [X] This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. [ ] This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. [X] This is an express request to promptly begin national examination procedures (35 U.S.C. 371(f)).
4. [ ] The US has been elected by the expiration of 19 months from the priority date (PCT Article 31).
5. [ ] A copy of the International Application as filed (35 U.S.C. 371(c)(2))
   a. [ ] is attached hereto (required only if not communicated by the International Bureau).
   b. [ ] has been communicated by the International Bureau.
   c. [ ] is not required, as the application was filed in the United States Receiving Office (RO/US).
6. [X] An English language translation of the International Application as filed (35 U.S.C. 371(c)(2)).
7. [ ] Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))
   a. [ ] are attached hereto (required only if not communicated by the International Bureau).
   b. [ ] have been communicated by the International Bureau.
   c. [ ] have not been made; however, the time limit for making such amendments has NOT expired.
   d. [ ] have not been made and will not be made.
8. [ ] An English language translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. [X] An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).
10. [ ] An English language translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11 to 16 below concern document(s) or information included:

11. [ ] An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
12. [X] An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
13. [ ] A FIRST preliminary amendment.
    [ ] A SECOND or SUBSEQUENT preliminary amendment.
14. [ ] A substitute specification.
15. [ ] A change of power of attorney and/or address letter.
16. [X] Other items or information: Specification with claims and Abstract.

JC18 Rec'd PCT/PTO 18 JUN 2001

U.S. APPLICATION NO. (if known, see 37 CFR 1.5)	INTERNATIONAL APPLICATION NO.	ATTORNEY'S DOCKET NUMBER
09/888608	PCT/UA98/000020	205.209

17. ☒ The following fees are submitted:	CALCULATIONS PTO USE ONLY
BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)) :	
Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO **$1000.00**	$ 1,000.00
International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO **$860.00**	
International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO **$710.00**	
International preliminary examination fee paid to USPTO (37 CFR 1.482) but all claims did not satisfy provisions of PCT Article 33(1)-(4) **$690.00**	
International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(1)-(4) **$100.00**	
ENTER APPROPRIATE BASIC FEE AMOUNT =	$ 1,000.00
Surcharge of **$130.00** for furnishing the oath or declaration later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492(e)).	$

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE	
Total claims	- 20 =		X $18.00	$
Independent claims	- 3 =		X $80.00	$
MULTIPLE DEPENDENT CLAIM(S) (if applicable)			+ $270.00	$
TOTAL OF ABOVE CALCULATIONS =				$ 1,000.00
☒ Applicant claims small entity status. See 37 CFR 1.27. The fees indicated above are reduced by 1/2.				$ 500.00
SUBTOTAL =				$ 500.00
Processing fee of **$130.00** for furnishing the English translation later than ☐20 ☐30 months from the earliest claimed priority date (37 CFR 1.492(f)). +				$
TOTAL NATIONAL FEE =				$ 500.00
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). **$40.00** per property +				$ 40.00
TOTAL FEES ENCLOSED =				$ 540.00
			Amount to be refunded:	$
			charged:	$

a. ☒ A check in the amount of $ 540.00 to cover the above fees is enclosed.

b. ☐ Please charge my Deposit Account No. _____ in the amount of $_____ to cover the above fees. A duplicate copy of this sheet is enclosed.

c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 01-0035. A duplicate copy of this sheet is enclosed.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

ABELMAN FRAYNE & SCHWAB
Attorneys at Law
150 East 42nd Street
New York, NY 10017
(212) 949-9022

June 18, 2001

SIGNATURE:

Jay S. Cinamon

NAME

24,156

REGISTRATION NUMBER

METHOD FOR TREATMENT OF PATIENTS USING EMBRYONIC CELL SUSPENSIONS

Field of the Invention

This invention relates to methods of treatment based on application of embryonic cell suspensions in treatment of human internal diseases when other modern methods and means have proven to be ineffective.

Proposed methods, hereinafter referred to as "cell therapy", may be used at the physician's discretion on clinical and outpatient basis depending on kind and severity of a disease.

Background of the Invention

Cell therapy comprises a relatively new branch of Transplántology. It is based on the use of suspensions prepared from living cells of cadaverous human embryos. Upon administration to the recipient's body, these cells are capable of engraftment, proliferation, and performance of the required functions.

Modern stage of cell therapy dates back to 1970's when this method started being considered as a possible alternative to bone marrow transplantation.

Thus, in 1973 administration of native cells of a fetal liver of a 7-week gestation for the first time resulted in the recovery of hematopoiesis in a patient with aplastic anemia (Kelemen E. *Second J. Hematol.*, 1973, v.10, No.4, pp.305-308).

Later on, administration of similar suspensions permitted to achieve positive results of treatment of primary and secondary conditions of myelodepression (Izzi T., Poletti O. et al. *Fetal liver transplantation, Alan R. Liss*, 1985, pp.237-249).

J.-L. Touraine used cell therapy for severe combined immune deficiencies caused by inborn genetic disorders (see e.g. *Transplantation Proceedings*, 1993, v.25, No.1, pp.1012-1013). Indeed, administration of genetically healthy pools of hematopoietic stem cells to children permits - especially in case of early (even at intrauterine stage) interference - to compensate immune defects.

Bacchetta R. et al (*J. Clin. Invest.*, 1993, v.91, March, pp.1067-1078) demonstrated remote results of cell therapy for severe combined immune deficiencies, when alongside with restoration of immunological indices, patients demonstrated signs of split chimerism and tolerance to both host and donor antigens.

However, to the authors' best knowledge, there were no publications disclosing cell therapy potential for treatment of numerous serious internal diseases in human patients, where other modern methods and means have proven to be ineffective. Moreover, the use of cell therapy for treatment of internal diseases is not mentioned even in such fundamental work as *Harrison's Principles of Internal Medicine* that was repeatedly published by McGraw-Hill in different countries and in different languages.

These aspects of the cell therapy were not addressed by the authors of the method of treatment of AIDS with embryonic cell suspensions, disclosed in the International Application PCT/UA94/00026 filed by A.I. Smikodub, I.S. Markov, and E.M. Pilipchak (see International Publication WO 95/16455, dated 06.22.95).

This method, being the most relevant to the claimed invention, comprises:

preparation of a suspension containing living embryonic cells selected from the group comprising hematopoietic liver cells, hematopoietic spleen cells, combination thereof, and pharmaceutically acceptable liquid medium, 1 ml of said suspension containing:

a) nucleated cells: $5\text{-}200*10^6$,

b) colony-forming units of granulocyte/macrophage (CFU-GM) $20\text{-}200*10^3$,

c) colony-forming units of granulocyte, erythrocyte, monocyte/macrophage, and megakaryocyte (CFU GEMM) $0.5\text{-}10*10^3$,
and
d) progenitor cells, CD_{34} (PC CD_{34}) $1\text{-}20*10^6$,
and at least single administration of such suspension to the body of an HIV-infected recipient, preferably in an amount of 0.5 to 5.0 ml.

Here, for such administration it is possible to use, either prepared *ex tempore* or frozen at cryogenic temperatures and subsequently thawed, suspensions that contain the above-indicated cells.

The above method has proven to be rather effective for treatment of AIDS. In all cases of its application, the period following administration of embryonic cell suspension may be divided into two stages that are clearly manifested and observed both by patients and physicians.

The first stage is represented by primary response that usually begins on the first day after administration of embryonic cell suspension, lasts for about one month, has progressing or undulating nature, is expressed by some decrease of clinical manifestations of the main disease and improvement of general condition and spirits of patients, which is accompanied by an increase of their mental and motor activity, being the most obvious in patients with severe forms of a disease.

The second stage is connected with manifestation of cell effects; it is usually develops in 1-1.5 months after administration of embryonic cell suspension and consists in the recovery of impaired functions of the recipient's body and in substantial decrease of clinical symptoms of the main disease.

The above method however has been oriented only at treatment of AIDS.

Summary of the Invention

Therefore, the main object of present invention is to develop - with due regard for kinds of diseases - by means of further improvement of the of embryonic cell suspensions prepared mainly from one human embryo, and by specific order of their administration to patients - such a method of treating human patients with the use embryonic cell suspensions that could be applicable for treatment of those human internal diseases that involve functional disorders, inflammatory and tumor processes, autoimmune aggression, dystrophy, and sclerotic changes manifested by weakness, fatigue and somatic depressions, where other modern methods and means have proven to be ineffective.

The object set forth has been achieved by the method of treatment of human patients with the use embryonic cell suspensions, providing:

preparation of a suspension containing embryonic cells selected from the group comprising hemopoietic liver cells, hemopoietic spleen cells, and a mixture thereof, and a pharmaceutically acceptable liquid medium; 1 ml of such suspension contains:

a) nucleated cells: $5\text{-}200*10^6$,
b) colony-forming units of granulocyte/macrophage (CFU-GM) : $20\text{-}200*10^3$,
c) colony-forming units of granulocyte, erythrocyte, monocyte/macrophage, and megakaryocyte (CFU GEMM) $0.5\text{-}10*10^3$,
and
d) progenitor cells, CD_{34} (PC CD_{34}) $1\text{-}20*10^6$,
and at least single administration of such suspension, prepared *ex tempore* or frozen at cryogenic temperatures and subsequently thawed, to the recipient's body, according to the invention, in addition to the above main suspension of embryonic tissues, at least one additional suspension is prepared, containing cells selected from the group consisting of liver stem cells of hematopoiesis, spleen stem cells of hematopoiesis, hepatocytes, thymocytes, epitheliocytes of the primary alimentary canal, nerve cells of brain, and mixes of cells of at least two said kinds,

and at least one such additional suspension is administered, along with the main one, to the patient's body.

As it will be demonstrated by way of Examples, in preparation and medical application, according to the invention, of at least two embryonic cell suspensions, an unexpected effect is manifested, consisting in extension of remission term, and in some cases, also in clinical recovery of some patients that have been considered incurable. It is astonishing that substantial improvements have been observed during treatment of patients suffering from neglected forms of such diseases as multiple sclerosis, primary cerebral dystrophy, circulatory insufficiency in aortal valvular disease, megacolon, Crohn's disease, and some others.

The first additional distinguishing feature consists in that at least one additional suspension is administered in the patient's body concurrently with the main suspension. With such procedure of administration, the synergistic effect is manifested as early as at the first stage of cell therapy, i.e. during the period between the first day and one month after beginning of treatment.

The second additional distinguishing feature consists in that prior to administration in the patient's body, at least one additional suspension is combined with the main suspension. Such procedure of administration simplifies manipulations and decreases consumption of time and auxiliary materials.

The third additional distinguishing feature consists in that the main and additional suspensions are administered successively in the patient's body. Such procedure of administration, with account of the progress of previous cell therapy and the forecast based on previous results obtained permits to select, out of the number of potentials, at least one such additional suspension that will provide additional augmenting and/or fixing of the attained curative effect.

The fourth additional distinguishing feature consists in that each additional suspension is administered in the patient's body after administration of the main suspension, during the period when the curative effect of previous manipulations usually reaches the maximum possible level. Here, with account of each previous stage of cell therapy and forecasts based on preliminary results obtained, it is possible to successively refine selection of additional suspensions for successive augmenting and/or fixing of the curative effect.

The fifth additional feature consists in that the main suspension is administered in the patient's body after administration of the additional one. Such a reverse procedure of administration of embryonic cell suspensions may be useful in cases where the compositions of additional suspensions can be rather closely related to recipient's body functions to be recovered, and therefore initial administration of the selected additional suspension can substantially predetermine the effect of the main suspension that is administered later.

The sixth additional distinguishing feature consists in that main and additional suspensions are prepared from tissues of the same embryo. This eliminates the competition between same types of cells from different embryos in the recipient's body.

Detailed description of the preferred Embodiments

The essence of the invention is further explained by:

disclosure of procedures of preparation of native embryonic cell suspensions,

disclosure of procedures of cryopreservation and storage of the above suspensions, and their thawing prior to administration,

disclosure of preferred methods of administration of cell suspensions to the recipient's body,

list of indications and contra-indications for application of cell therapy of the invention,

summarized description of curative effects achieved by application of at least one main and at least one additional embryonic cell suspensions, and

Examples of application of cell therapy for treatment of hard-to-cure diseases.

Preparation of embryonic cell suspensions includes:

a) laying embryos of 5 to 14, and preferably 5 to 12-week gestation, obtained through legal abortions in healthy women, into sterile Petri dishes filled witha sterile and pharmaceutically acceptable nutrient medium, e.g. standard Hanks's solution with addition ofan antibiotic, preferably of the aminoglycoside family, in particular - gentamycin in the concentration of about 160 mg/l of nutrient medium; or McCoy 5A medium to which antibiotic is also added (usually a combination of penicillin and streptomycin), and which is modified by additives of basic and non-basic amino acids, sodium pyruvate,a complex of vitamins, and serine-asparagin-glutamine mixture;

b) preparation of cadaverous embryos inside a sterile box in order to separate the organs whose cells will be used for suspensions, and namely:

* liver and spleen that are used, either separately or in combination:

** in all cases for preparing the main medical suspension of hemopoietic cells, and

** in some cases: for isolation of hematopoietic stem cells and (only from liver) hepatocytes, the latter being the active part of at least one medicinal suspension;

*thymus, primary alimentary canal, and brain: also to prepare at least one additional medicinal suspension whose active part, as it has been mentioned,contains cells selected from the group consisting of stem cells of liver hemopoiesis, stem cells of spleen hemopoiesis, hepatocytes, thymocytes, epitheliocytes of the primary alimentary canal, brain nervour cells, an combination of cells of at least two above-mentioned types, and

* musculocutaneous graft for preparation of control cell suspensions for viral testing;

c) fragmentation and preparation of suspensions from selected organs by application of common methods and facilities and, if required, selection of the required type cells from their total mass;

d) packaging of medicinal and control suspensions into sterile, hermetically sealed 0.5 to 2.0 ml (depending on further purpose) PE containers, and labelilng of the containers.

Hanks's solution used for preparation of embryonic cell suspensions must have pH level ranging from 7.2 to 7.4. In order to achieve the required pH value, added to this solution is sterile 1.4% $NaHCO_3$ solution and the required pH value is determined from the orange-red color of samples when adding the phenol red indicator. Shelf life of sterile Hanks's solution before use at temperatures from +4°C to room temperature is 1 month.

Similarly, modified McCoy 5A medium must have pH of 7.0-7.1. Provided standard package of the dry McCoy 5A contains 2.2 g of it, used are 8 ml of MEM solution of basic amino acids and 4 ml of MEM solution of non-basic amino acids, 10 ml of MEM solution of sodium pyruvate and 4 ml of MEM vitamin solution, 420 mg of L-serine, 800 mg of L-asparagin, and 200 mg of L-glutamine.

For subsequent medical application prepared were the following embryonic cell suspensions:

a) two basic (main) suspensions, namely:

No.1: suspension of hematopoietic liver cells;

No.2: suspension of hemopoietic spleen cells, and

b) six additional suspensions, namely:

No.3: suspension of hematopoietic liver stem cells;

No.4: suspension of hematopoietic spleen stem cells;

No.5: suspension of hepatocytes;

No.6: suspension of thymocytes;

No.7: suspension of epitheliocytes of the primary alimentary canal, and

No.8: suspension of brain nervous cells.

Preferred dispersion media include:

for suspensions Nos 1, 2, 6, 7, 8: - Hanks's solution, and

for suspensions Nos 3, 4, 5 - McCoy 5A medium.

Suspensions Nos 1, 2, 6, 7, 8 were prepared in homogenizers from relevant embryonic tissues, filtrated through standard filter used for transfusion of blood preparations,blood transfusions, and then through a series of needles with decreasing diameters, while cell material required for preparation of suspensions Nos 3, 4, 5 was obtained by repeated centrifugation and removal of supernatant.

The above suspensions may be used in the native form within 6 to 8 hours from the moment of obtaining an embryo and in case of storage at +5-7°C prior to their administration to a recipient.

The use of cryopreserved embryonic cell suspensions is however preferable since, according to our experience and data provided by other researchers (see e.g. Ek S., Rindgen O., Markling L., Westgren M. Cryopreservation of fetal stem cells. Bone Marrow transplantation, 11 Suppl., 1:123, 1993), they are practically similar to native ones in terms of composition and clinical effectiveness, while being much more convenient as for selection of recipients and terms of use. According to the method of invention, native suspensions must be used only once, during the first curative session.

Control of cell suspensions prepared from each embryo has been carried out by mandatory use of

one 0.5 ml container for testing functional validity of each medicinal cell suspension upon thawing;

three ≤2.0 ml containers, containing 1.0-1.5 ml of control cell suspension prepared from musculocutaneous graft and intended for viral testing (the first is intended for detecting dangerous viruses at the laboratory where cell suspension are prepared; the second container is intended for similar parallel testing at the state control laboratory; the third container is for storage at the cryobank and subsequent confirmation of safety of embryonic material used in case any disputes arise);

two containers of up to 2.0 ml, each containing 1.0 to 1.5 ml of rinse water from laboratory utensils and instruments for bacterial sterility testing (contents of the first container are tested at the producer's laboratory, and contents of the second container - at the state control laboratory).

The above controlling measures, combined with testing embryo donors for syphilis, HIV infection, HBV and HCV, toxoplasmosis, cytomegalovirus, testing of embryos for HIV infection, HBV and HCV, toxoplasmosis, cytomegalovirus, viruses of rubella and herpes, and with repeated testing of donors for HIV infection 90-100 days after abortion ensure safety of cell therapy of the invention.

In case of detection of infectious pathogens in the producer's laboratory, all the material prepared from the infected embryo was destroyed by incineration together with containers.

Prior to application of medicinal embryonic cell suspensions at the clinic, by commonly accepted methods determined were concentrations of CFU-GM, CFU bl., and PC CD_{34} in 1 ml of nucleated cel . Such full-scale procedure is mandatory for cryopreserved cell suspensions Nos 1, 2, 3, and 4, while for other suspensions and for native suspensions Nos 1, 2, 3, and 4, provided they were prepared in compliance with the above-described standard procedure, it is allowed to determine concentrations of nucleated cells only.

The required concentrations were determined:

for nucleated cells - with the use of cell analyzer or visually, under a microscope, in a counting chamber;

for CFU and CFU bl. - by methods of CFU cloning in methyl cellulose (see Hann V., Bodger M., Hoffbrand A. Development of pluripotent hematopoietic progenitor cells in the human fetus//*Blood*, 1983, Vol.62, No.4, pp.118-123);

for CD34 - by indirect immune fluorescent test with monoclonal antibodies in a flow-type cytofluorimeter.

Cryopreservation of embryonic cell suspensions was carried out in three stages, similar to the procedure disclosed in Fedotenkov A.G. et al. Cryopreservation of bone marrow at low temperatures for clinical purposes//*Problemy Hematologii*, 1966, Vol.10, No.2, pp.45-50, using as cryoprotector sterile dimethylsulfoxide (DMSO) in the final concentration of 3- 10% of the amount of cell suspension.

Containers with embryonic cell suspensions to be frozen were placed in a vertical position inside the chamber of a programmable freezer and frozen:

a) from room temperature to –4°C - at the rate of 1°C/min;

b) from -4°C to -10°C - at the rate of 0.1°C/min;

c) from -10°C to -196°C - at the rate of 7°C/min.

Storage term of containers with embryonic cell suspensions in liquid nitrogen is practically unlimited.

Prior to application, embryonic cell suspensions from cryobank were thawed in two stages:

First stage: quickly, in a water bath at 40 °C till the appearance of a small moving piece of ice in the center of the container.

Second stage: slowly, in the air, at room temperature, till disappearance of the above-mentioned piece of ice in the container.

Prior to administration, thawed cell suspensions may be stored at room temperature for no longer than 2 hours.

It has to be noted, that removal of DMSO from thawed cell suspensions is not required, since for safety purposes its dose, even in case of maximal (about 8 ml) single administration, is substantially lower than the permissible amount. Moreover, DMSO, being a conductor of present in cell suspensions biologically active substances through biological barriers and membranes, stipulates higher efficiency of cell therapy.

Medicinal cell suspensions can be administered to the recipient's body in different ways.

Preferable is - particularly for main suspensions Nos 1 and 2 - intravenous administration, together with about 100 ml of isotonic solution, at the rate of 20-40 drops per minute.

Also possible - especially in children - is jet-type intraperitoneal administration of cell suspensions diluted with isotonic solution to the amount of ~ 50 ml.

In cases of fresh thrombi, hemophthalm or hypersplenism, the most expedient is intraosteal (preferably in breast bone) jet-type administration of cell suspensions diluted with isotonic solution to the amount of ~ 50 ml.

Often expedient is subcutaneous administration, preferably of additional suspensions Nos 3-8.

Finally, it is also possible to create a depot of embryonic cells in cavities and fasciae of the recipient's body.

Amounts of simultaneously administered doses of medicinal suspensions may be chosen within the range of 0.5-8.0 ml, preferably ~5.0 ml. It should be however born in mind that:

there is no direct connection between the increase of the dosages and either manifestation or stableness of the curative effect; therefore, high (5-8 ml) dosages are recommended only in cases where administered embryonic cells have to provide maximum possible manifestation of the first stage of cell therapy,

administration of less then 0.5 ml dosages is technically inconvenient, because part of cells always remain in flasks, tubes, needles etc., and

taking into consideration all the above-mentioned, it is usually enough to administer from 0.5 to 2.0 ml of main and additional suspensions each during one session of cell therapy.

During repeated sessions of cell therapy at subsequent stages of treatment, it is preferable to use cell suspensions prepared from embryonic material that was used for the first session. That is why prepared suspensions are poured out into several containers.

Indications for cell therapy of the invention generally include (but are not limited to)

diseases proceeding with expressed disturbances of the blood system;

tumor diseases, with the purpose of:

*initial treatment that involves removal of the tumor, in cancer complicated by anemia, fever, cachexia, concomitant infections etc.

*carrying out of chemotherapy and radiotherapy courses, and

*formation of anti-tumor immunity during remission;

diseases resulting in the development of connective tissue (sclerosis);

diseases with underlying inflammatory processes;

diseases whose progress causes ulcerous and erosive defects;

diseases progressing against the background of chronic infections;

diseases, major manifestation of which is dystrophic process;

diseases based on functional disorders in the form of uncoordinated, untimely, dystonic functioning of organs, parts and systems of organs;

autoimmune and allergic disorders;

resistance to medicinal preparations that are vital for patients, and

ineffectiveness of commonly accepted methods of treatment.

Cell therapy of the invention is contra-indicated:

in pronounced hypertension of lesser circuit, with concomitant development of acute or sub-acute *cor pulmonale*;

in myelocarsinosis,

at terminal stages, of mainly oncological diseases, with strongly expressed deep intoxication, severe metabolic disturbances, and serious general decompensation, and

in acute forms of vasculitis, capillaritis, phlebitis, arteritis, and thrombosis.

However, by three months of remission after active treatment of acute forms of vasculitis, capillaritis, phlebitis, arteritis, and thrombosis, cell therapy according to the invention is quite possible. It is also natural that prior to the beginning of cell therapy, sanitation of the foci of chronic infections is expedient.

Main curative effects that can be achieved by cell therapy according to the invention include:

restoration of such immunity indices as total lymphocyte count, including subpopulations of T3, T4, and T8 lymphoctytes, NK cells and B-lymphocytes, and normalization of their ratio; termination of autoimmune aggression and immune suppression, and hence restoration of anti-tumor and anti-infection immunity;

restoration of such blood indices as leukocyte, erythrocyte, and thrombocyte counts;

decrease of frequency of hemorrhages episodes;

normalization of functional activity of organs, parts and systems of organs;

recovery of lost functions of tissues and organs;

decrease of symptoms related to formation of connective tissue;

improvement of trophicity of organs, improvement of microcirculation, plethora in tissues and organs;

intensification of reparation processes, manifested in healing of ulcerous and erosive defects;

improvement of psychoemotional condition of patients, strengthening of their will for recovery, reduced anxiety and fears;

decrease of symptoms of intoxication: weakness, sweating, reduced capacity for work, sleep disorders and appetite disturbances;

anesthetic action, and

restoration of sensitivity to medicinal preparations.

Examples given below are intended to explain the method of joint application of at least one main and at least one medical suspensions, additional medicinal suspension, and synergistic effects achieved thereby. It is however evident that these Examples do not cover all possible applications, and curative effects that can be achieved.

Example 1

Patient A., male, born in 1944, was admitted to the Cell Therapy Clinic on May 10, 1994, with the following diagnosis: aortic incompetence, consequences of chest and heart contusions (December 1990, car accident), circulatory insufficiency, mixed type FC 3, pulmonary edema (April 11, 1994), atrophic stomatitis, glossitis, gingivitis.

The patient considered himself ill since 1992 , i.e. the time when there developed the initial signs of heart disease secondary to chest and heart contusions: wincing of the head, decrease of the diastolic blood pressure, palpitation. In 1993, the patient was offered surgical treatment that was rejected for religious reasons. Since 1994, he has been constantly taking cardiac glycosides, diuretics and inhibitors of angiotensin-transforming factor. In the evening of April 11, 1994, pulmonary edema started; it was cupped off by the ambulance crew. The patient was admitted to the intensive therapy unit of the City Cardiological Center. Later on, he was treated in the Department of Cardiology . Despite prescribed therapy, the patient complains of dyspnea at insignificant loads, worsening at night; edemata of lower extremities; palpitation, pain and heaviness in the right hypochondrium, as well as pain in mouth, xerostomia, inability of chewing food. The patient is depressed by his condition.

On examination: male, height - 182 cm, weight - 90 kg, skin is pale, visible mucous coats are cyanotic. Carotid pulsation on the neck; pulsation in the jugular fossa. Synchronous wincing of head and capillary pulse. Pulse - 88 beats/min, rhythmic, blood pressure (BP) 140/30. Intensified and expanded apex beat in the sixth intercostal area along the anterior midaxillary line. At percussion, the borders of the relative and superficial cardiac dullness are considerably shifted to the left. At auscultation, the melody is typical for mitral incompetence. First heart sound is reduced; second heart sound is considerablyly weakened; diastolic murmur mild and blowing. Independent systolic murmur at the *apex cordis*.

Percussion sound above the lungs is clear and weakened in lower segments of both lungs. Lung fields are clear, breath is weakened. Isolated dry rales. Crepitation in the lower sections of the lungs. Abdomen is soft and tender in the right hypochondrium; liver - by 10-12 cm below the edge of the costal arch, the liver edge being sharp and tender. Intestinal segments are normally positioned and have usual properties. Expressed dense pododema, crus edema, and hydroscheocele.

Examination of the oral cavity revealed "varnished type" membranes, thinned and hyperemic; observed are several cracks covered with fibrin; gingiva is hemorrhagic, edematic and hyperemic; the tongue is smooth, without papilla; small erosions along edges of the tongue in the foci of teeth imprints. Perleches.

Diagnosis has been confirmed by X-ray and ultrasound examination of chest organs, heart, and major vessels.

Noteworthy is patient's resistance to cardiac glycosides and saluretics. Despite diet rich in potassium and treatment, it was impossible to decrease manifestations of circulatory insufficiency. Diurnal diuresis was still less than 700 ml. Positive diuresis was absent.

May 12, 1994 – administration of :

a) suspension No.2 (sample S-214L; cell count - $100*10^6$/ml; CFU-GM – $82*10^3$/ml; CFU bl. - $3.2*10^3$/ml, and PC CD_{34} - $8*10^6$) intravenously, dropwise, in the amount of 2.0 ml;

b):suspension No.5(sample S-214H; cell count - $8.4*10^6$/ml), subcutaneous, in the amount of 1.5 ml, and suspension No.7 (sample S-214E; cell count - $43*10^5$/ml), in two upper and two lower quadrants of anterior abdominal wall, in the amount of 1.5 ml,.

By the evening, the patient noted intensification of diuresis and decrease of dyspnea in the state of repose. At night, he slept quietly for the first time in last few months. Next morning he was cheerful; dyspnea subsided; the patient noted disappearance of pain in the oral cavity and right hypochondrium. During the day, he urinated frequently. Diurnal diuresis amounted to 2.5 l. Within a week, edemata of lower extremities disappeared, liver size decreased. Its edge was palpated painlessly, 3 to 4 cm below the costal arch. Lung fields were clear, dullness in lower segments disappeared. Blood pressure rose to 170/20. Dyspnea at walking substantially decreased. He started sleeping quietly, without any asphyxia. Pain in the oral cavity, caused by chewing food, disappeared as well as perleches; bleeding of gingiva decreased. The patient started brushing his teeth again.

Digitalis and saluretic preparations were considered once again, in new dosages. Positive diuresis was noted every day. The patient was discharged from the clinic on May 22, 1994.

During control examinations, once in every two months, it was noted that the patient increased daily loads, started shopping and cleaning the house; his mood became even. Optimism appeared; he began to follow doctor's prescriptions, limited consumption of salt and liquids. There were no visible edemata. The liver edge - by 2 cm below the edge of the costal arch. Three months later, mucous membranes of the oral cavity became velvety; there were no erosions, cracks, or bleeding. Mucous coats of the tongue were restored. Dosages of Digitalis and saluretic were decreased by 50%.

Ultrasound examination of heart on October 10, 1994 revealed a 37% increase of contractile ability of myocardium.

The patient was observed in the clinic during 1994-1996. He received similar therapy with embryonic cell suspensions in September 1996. In 1997, he moved to another country.

Example 2

Patient P., male, born in 1981,was admitted to the Cell Therapy Clinic on November 16, 1994, with the diagnosis of acquired hypoplastic anemia. On admittance, he complained of headache, vertigo, palpitation, dyspnea at insignificant loads, poor appetite, expressed weakness, and hemorrhagic eruption mostly along lateral surfaces of the extremities.

The disease started 2 months ago; he received corticosteroids – Prednisolone, 60 mg daily without any positive effect. Received 800 ml of erythrocytes weekly via transfusions.

On examination: overweight, dysplastic adiposis in places typical for Cushing's syndrome. Skin is pale, abundant spotted petechial eruption on the crus skin. Peripheral lymph nodes are not enlarged. Pulse - 104 beats/min, rhythmic, full and tense. Blood pressure - 110/70. Borders of the relative cardiac dullness are within normal limits. Heart sounds are regular; systolic murmur at the *apex cordis*. Percussion sound above lungs is clear. Lung fields are clear. At palpation, abdomen is soft and indolent. The lower edge of liver protrudes by 2 cm beyond the edge of the costal arch along the right midclavicular line. Spleen is not palpated.

General laboratory tests and instrumental tests were carried out in full scope and dynamics. Blood tests were performed; in order to specify the diagnosis, sternal puncture was performed on November 18, 1994 (Tables 2.1 and 2.2).

Myelogram revealed considerable oppression of myeloid and erythroid cell lines and practical absence of megakaryocytic cell lines.

Diagnosis: acquired aplastic anemia.

November 20, 1994 – administration of suspension No.3 (sample C-861SH; cell count – $42*10^3$/ml; CFU-GM - $31*10^3$/ml; CFU bl. - $26*10^3$/ml, and PC CD_{34}-$29*10^3$/ml), intravenously, dropwise, in the amount of 2.5 ml.

In 8-12 hours after administration, the patient noted the improvement of general condition, appearance of appetite, reduced weakness, headache was not so intense. There was no fever. On the first day after administration, observed was insignificant increase of the level of erythrocytes and hemoglobin, and on the seventh day - increased leukocyte count (Table 2.1). Thrombocyte count did not increase. Myelogram performed on the 9th day after the first transplantation revealed insignificant increase of amounts of all hematopoietic cell lines. Beginning from the third week after administration of suspension No.3, indices of peripheral blood started to decrease (see Table 2.1).

Repeated administration of the same suspension No.3 intravenously, dropwise, in the amount of 1.4 ml. Administered additionally were:

suspension No.4 (sample C-861SL; cell count - $18*10^3$/ml; CFU-GM - $10.2*10^3$/ml; CFU bl. - $12*10^3$/ml, and PC CD_{34} - $5.6*10^3$/ml), intravenously, dropwise in the amount of 2.0 ml

suspension No.1 (sample C-861; cell count - $124*10^6$/ml; CFU-GM - $52*10^3$/ml; CFU bl. - $4.0*10^3$/ml, and PC CD_{34} - $2.34*10^6$/ml), subcutaneous, in anterior abdominal wall, in two depots, in the amount of 1.5 ml each;

suspension No.6 (sample C-861T; cell count - $74*10^4$/ml), intravenously, dropwise, in the amount of 2.0 ml.

On the third day after the second administration, erythrocyte count decreased to $1.5*10^{12}$/l. Further increase in the amount of blood-forming elements of different cell lines was accompanied by clinical improvement of patient's condition: no new eruption on skin, decrease of intoxication (weakness, sweating), appearance of appetite. Dosages of glucocorticoids were actively reduced; no transfusions of blood components were carried out. Considerable increase of thrombocyte count in the peripheral blood was noted on the 7th day; subsequently, tendency of its increase was observed until normalization. . Increase of leukocyte count in the peripheral blood was observed starting from the 30th day after the second administration, with the tendency to steady increase. Erythrocyte count was restored much slower. Within 50-60 days, its range was $2.5\text{-}3.0{\cdot}10^{12}$/l; hemoglobin - 75-90 g/l. Increase of red blood cell count with a tendency to complete normalization was observed during 3- 4 months after the second administration.

Myelograms performed on the 15th and 34th days after the second administration, demonstrated the improvement of hematopoiesis of all bone marrow cell linces, including megakaryocyte cell lines (see Table 2.2).

- At present, observation is continued.

Example 3

Patient D.M., male, born in 1936, a lawyer, was treated at the Cell Therapy Clinic from May 16, 1996 till May 20, 1996.

According to his daughter, his illness started in March 1994. Members of his family and his colleagues noticed inadequate responses of the patient; documents, prepared by him, were of poor quallity, his handwriting changed, he could not easily participate in discussions and state his ideas clearly. The patient started losing memory about recent events; he became sluggish, depressed, and emotionally inert; his gaze became fixed.

Since 1994, main observation was made by psychiatrist. In 1996, the above phenomena worsened, which was manifested by tangled, staccato speech; he could not write or sign documents; "cut-off" periods lasted for more than 10 minutes, after which he was unable to find himself in usual environment. During the daytime, he often fell asleep while sitting in an armchair.

In April 1996, cerebral blood flow with radioactive Xe^{133} and computer tomography performed at Harbor/UCLA Diagnostic Imaging Centre and Good Samaritan Hospital, allowed to diagnose an atrophic process in orbitofrontal, dorsofrontal, and temporal regions of left hemisphere of the brain, as well as in the right cerebellar lobe.

During the last month, the patient started falling while stepping over small hights, like threshold, and sometimes for no reason. His gait became uncoordinated and unsteady. The patient received 1.2 g of Pyracetam daily and 10 mg of Diasepam, before sleep.

. On examination: pale, tall, athletic man with a mask-like face, giving one-word answers to the questions. He often answers irrelevantly, or changes his answer or subject of discussion. Skin is clean, lymph nodes are not enlarged. Pulse - 66 beats/min; blood pressure - 130/80, respiration rate - 16/min. Internal organs are intact.

May 16, 1996: Laboratory tests. Results of the total blood count and blood immunology are given in Tables 3.1 and 3.2, respectively. May 16, 1996: Blood chemistry: total bilirubin - 14.0 mmole/l; direct – negative, indirect - 14.0 mmole/l; thymol test - 2.0 units; ALT - 0.31 mmole/l; AST - 0.22 mmole/l; cholesterol - 3.3 mmole/l; creatinine - 0.135 mmole/l; urea - 12.5 mmole/l; total protein - 76.3 g/l; albumins - 54.1 %; globulins - 45.9 % (including: αl1 – 5 %; α2 – 9.9 %; β– 13.7 %, and γ globulin – 17.3 %); seromucoid - 0.185 units.

May 16, 1996: ECG – rhythm is sinus and regular. Signs of moderate changes of myocardium.

May 18, 1996: Examination by Ophthalmologist. Findings: frontal segment without changes. Optical parts are transparent in the passing light. Eyeground: disks of the optical nerve are contoured, pale-pink. Arteries are narrowed, sclerotic. Rings of peripapillary atrophy of choroid. Macular areas without focal pathology. Diagnosis: medium-degree myopia, angiosclerosis of the retina OU.

May 18, 1996: Examination by Neurologist. Findings: complaints of mental changes, manifested at performing functional activities; occasional motor ataxia. The patient has been ill for about 4 years. Looks older. Family history: father had cancer of large intestine; unremarkable for mental diseases. Neurological status: eye slits, pupils d=s, full-range motion of eyeballs. Ny – none; left nasolabial fold is smoothed; tongue is in the midline. Swallowing is intact. Full-range active movements, strength is normal, muscular tension without changes, medium-vivacity reflexes, d=s, Strumpel's sign on both sides.

Coordination tests from the left are performed clumsily; in sensibilized station test (Romberg's position) falls to the left. Sensibility to pain is preserved. Moderately expressed adiadochokynesis on the left; Babinski's asynergia is absent. Thus, symptoms observed in the patient indicate damaged brain formations on the left. It is necessary to carry out differential diagnostics between (+) process and idiopathic cerebral atrophy. It is expedient to perform contrast angiography and motor examination of the eyegrounds.

Diagnosis: idiopathic encephalopathy, involution of the left part of the frontal lobe, frontal part of the temporal lobe, and right hemisphere of the cerebellum; initial manifestations of dementia; ataxia.

May 16, 1996 – administration of suspension No.1 (sample 3038AP284; cell count - $189*10^6$/ml; CFU-GM - $42*10^3$/ml; CFU bl. - $7.6*10^3$/ml, and PC CD_{34} - $3.2*10^6$/ml) intravenously, dropwise, in the amount of 1.0 ml .

Everybody who accompanied him noted that by the evening time the patient became more animated: he laughed, watched TV for a long time, and was carried away by the show, which had not happened to him for a long time. He took 2 pills of Diasepam. The patient slept all night long. Next morning he was animated, had a good breakfast. He was talking to his daughter all day long.

May 17, 1996 – administration of suspension No.8 (sample 3038AM284; cell count - $64*10^6$/ml), subcutaneous, in anterior abdominal wall, in two depots in the amount of 1.5 ml each.

May 18, 1996 - repeated administration of the same suspension No.1, intravenously, dropwise and additional subcutaneous administration of same suspension No.8 in anterior abdominal wall, in two depots in the amount of 1.5 ml each.

On May 19 - 20, 1996, the patient started walking better; shaking disappeared; he was able to pass the station test, did not fall a single time. He became more animated and talkative; there appeared facial expression. Appetite has improved. He was able to sign a financial document. Recommendation: a course of treatment with Vinpocetinum (1x3 times a day for 2 months), visit to the Clinic in July 1996.

Patient D.M., accompanied by his son, stayed at the Clinic from July 1 till July 5, 1996.

Diagnosis was the same: idiopathic encephalopathy, involution of the left part of the frontal lobe, frontal part of the temporal lobe, and right hemisphere of the cerebellum; initial manifestations of dementia; ataxia.

During examination it was found out that the patient had returned to his regular life. He works for his office but does not speak in public. Practices exercise, running, swimming; drives a car. Started denying the fact of his former illness.

On examination: animated, active, athletic man. Gives adequate replies to questions; though his answers are still somewhat inert, some sounds are unclear, observed some extent of staccato speech. His writing has changed.

July 1, 1996: Laboratory tests. Results of the total blood count and blood immunology are given in Tables 3.1 and 3.2, respectively. Blood Chemistry: total bilirubin - 15.0 mmole/l; direct – negative, indirect - 15.0 mmole/l; thymol test - 2.5 units; ALT - 0.45 mmole/l; AST - 0.24 mmole/l; cholesterol - 6.0 mmole/l; creatinine - 0.130 mmole/l; urea - 12.58 mmole/l; total protein - 80.6 g/l.

July 1, 1996: ECG – rhythm is sinus, regular.. Signs of moderate changes of myocardium.

Taking into consideration the effectiveness of previous treatment, repeated course of cell therapy was recommended.

July 1, 1996 – administration of suspension No.4 (sample 3038ALS284; cell count - $24*10^3$/ml; CFU-GM - $19*10^3$/ml; CFU bl. - $12.6*10^3$/ml, and PC CD_{34} - $7.1*10^3$/ml). intravenously, dropwise, in the amount of 3.0 ml.

July 2, 1996: Examination by Neurologist. Findings: according to the people around, the patient's condition has improved; no complaints were made during examination. On examination: no focal organic changes of the nervous system were detected; he was stable during the station test; no adiadochokinesia was present. Gait is unremarkable, synkinesia of arms at walking is satisfactory.

July 2, 1996 - additional administration of suspension No.8 (sample 3038AM284; cell count - $64*10^6$/ml), subcutaneous, in anterior abdominal wall, in two depots, in the amount of 1.5 ml each.

After treatment, the patient once again noted an increase of physical activity and improvement of general condition.

Later on, the patient contacted the Clinic's experts and informed them about his satisfactory condition. In 1997, he participated in a Marathon race in Los Angeles.

Example 4

Patient D.S., male, born in 1959, lawyer, was treated at the Cell Therapy Clinic from October 6, 1996 till October 11, 1996.

According to the patient, the disease started in 1993. At the beginning of that year, sensibility of some fingers disappeared for 15 days and then came back spontaneously. In July 1993, after respiratory infection, he felt weakness in his legs, his gait was shaky, there were also coordination problems and occasional fevers. During examination at the hospital, no causes of

such condition were detected. Treatment with Hydrocortisone was successful, symptoms of disease disappeared.

In February 1994, weakness in legs and problems of walking reappeared; observed were fevers and an increase of leukocyte count to 12 g/l were noted. The patient was tested for AIDS, and consulted by numerous specialists. In March 1994, brain scanning at the Princess Grass Hospital (Monaco) revealed degeneration foci in different sections of brain.

The diagnosis was: Multiple sclerosis.

The patient was treated with Glucocorticoids and Interferon. Since the end of 1994, he had been using a wheeled chair. Expressed muscular hypertension of upper and lower extremities. The patient is not capable of self-service. He is limited in his work activities, occasionally, he is unable to hold a pen in his hands, and complains of expressed general weakness. Limited in his contacts with other people. There appeared problems with urination. Sexual activity is impossible.

On examination: male sitting in a wheel chair, handsome. Offers his hand for greeting, with difficulty, almost without squeezing. In order to carry out examination, he was assisted to bed after fixing his hyper tense lower extremities. The patient was undressed by medical staff. Skin is clean and pale, normal complexion. Has two poorly formed fingers (from birth) on the left hand. Squint (surgery at the age of 12). Physical examination revealed no changes of internal organs, heart, lungs, gastrointestinal tract, liver, and kidneys. No edemata is observed. Pulse - 72 beats/min; blood pressure - 120/70, symmetric. Respiration rate - 18/minute.

Diagnosis: Multiple sclerosis, cerebrospinal form with tetraparesis, d>s, moslty in legs. Divergent concominant squint OS. Middle-swinging nystagmus. Hypermetropic astigmatism. Angiopathy of retina. Beginning macular degeneration OU.

October 7, 1996 :Laboratory tests. Results of total blood count and blood immunology from October 7, 1996 are given in Tables 4.1 - 4.2.

Blood Chemistry: total bilirubin - 17.6 mmole/l; direct – negative, indirect - 17.6 mmole/l; thymol test - 4.3 units; ALT - 0.32 mmole/l; AST - 0.21 mmole/l; cholesterol - 5.4 mmole/l; creatinine - 0.065 mmole/l; urea - 0.62 mmole/l; total protein - 74.2 g/l; blood sugar - 4.6 mmole/l.

Urinanalysis: amount - 180.0 ml; straw color, transparent, acidic; sugar – neg; protein – neg; squamous epithelium – small amount; leukocytes - 2-3 in the field of microscope; relative density - 1018; mucus - moderate amount; oxalates - small amount.

ECG – rhythm is sinus and regular. Position of the heart electrical axis is normal. Dystrophic changes of myocardium.

Examination by Neurologist. Findings: complaints of inability to walk – "heavy", "chained" legs; the patient can stand only with somebody's assistance because of ataxia and weakness in legs. The patient also complains of visual disturbances: convergence is problematic, thereby causing duplemia (attempted fixation leads to outward (outer corner) flow of the left eye). The disease started 4 years ago. Neurological status: contactable, oriented; divergent strabismus caused by the left eye. Pupils d=s, dull photoreactions. Middle-swinging horizontal nystagmus at looking to the side, s>d. The face is symmetric, tongue in the midline; gluttural reflex is preserved. Range of active motions in arms and legs is limited; tension in arms is slightly increased; expressed spasticity in legs; high reflexes, d>s; foot clonus d>s; "fan" symptom on both sides. Sensibility to pain is preserved; coordination tests are performed satisfactory. Results of MRI of the brain: multiple degeneration foci ("patches") in *truncus cerebri* (cerebellum and other parts), *corpus calossum*.

Recommendations of ophthalmologist: eyegrounds, *visus*, fields of vision.

October 10, 1996: Urinanalysis: amount - 70.0 ml; straw color, transparent, acidic; sugar – neg; protein – neg; squamous epithelium – small amount; leukocytes, 1-3 in the field of microscope; relative density - 1018.

October 11, 1996: Blood sugar - 3.3 mmole/l.

October 11, 1996: Examination by Ophthalmologist: preliminary test of vision in spectacles >0.6. Refraction: hypermetropic astigmatism. Full range of motions of eyeballs. Divergent squint OS (10-15 degrees on Girschberg scale). Middle-swinging horizontal nystagmus at looking sideways. Lively reaction of pupils to the light. Frontal segment is intact. Optical parts are transparent. Eyegrounds: disks of optical nerve are contoured, pale-pin. Arteries are narrowed. Arteries/veins=1/3. Veins are twisted. Isolated foci of dystrophy in the macular area and central zones of the retina.

Diagnosis: Divergent concominant squint OS. Middle-swinging nystagmus. Hypermetropic astigmatism. Angiopathy of retina. Initial maculodystrophy of both eyes.

October 7, 1996 - administration of suspension No.1 (sample 3038AP118; cell count - $184*10^6$/ml; CFU-GM - $68*10^3$/ml; CFU bl. - $4.3*10^3$/ml, and PC CD_{34} - $7.2*10^3$/ml) intravenously, dropwise, in the amount of 2.0 ml; additional administration of suspension No.8 (sample 3038AM118; cell count - $89*10^6$/ml), subcutaneous, in anterior abdominal wall, in two depots, in the amount of 0.7 ml each.

Several hours later, the patient noted a decrease in the tension of the extremities; his movements became more coordinated. At night he made several attempts to stand up on his own, and succeeded. Next morning he felt inspired. He noticed strength in his body, and noted that he was able to hold various objects better. He demonstrated ability to cross his legs while sitting in an armchair. The patient noted improvement of his appetite. He also noted the improvement of the ability to hold urine prior to urination. He called his home country, dialing telephone number himself.

October 9, 1996 - repeated administration of the same suspension No.1, intravenously, dropwise; additional administration of the same suspension No.8, subcutaneous, in anterior abdominal wall, in two depots in the amount of 1.5 ml each.

At the time of discharge from the Clinic, it was noted that patient's condition had improved. Weakness in upper and lower extremities decreased, and muscular hypertension of the neck, of the back, and of both lower extremities has decreased. Divergence of eyeballs has reduced. Sleep has normalized; appetite and spirits improved. Capacity for work has increased. Recommendations: visit to the Clinic within the period of December 1-6, 1996.

Diagnosis is the same: Multiple sclerosis, cerebrospinal form. Divergent concominant squint OS. Middle-swinging nystagmus. Hypermetropic astigmatism. Angiopathy of the retina. Initial maculodystrophy of both eyes.

The patient tells about positive dynamics. He has endured the flight well. The patient is talkative. He took off his jacket, shirt, and underwear without assistance. Hypertension of lower extremities disappears much faster when he changes position of his legs. The patient brought a PC for work and books for reading. During the day, weakness does not bother him; he sleeps and eats well. Sexual activity reappeared. The patient believes that he does not have any problems with urination. On examination: internal organs are intact. Pulse – 76-78 beats/min; blood pressure - 115/70, respiration rate - 16/min.

Laboratory tests. Results of the total blood count from December 2 and 4, 1996 are given in Table 4.1, and blood immunology indices from December 2, 1996 - in Table 4.2.

: total bilirubin - 21.7 mmole/l; direct – negative, indirect - 21.7 mmole/l; thymol test - 1.7 units; ALT - 0.34 mmole/l; AST - 0.21 mmole/l; creatinine - 0.062 mmole/l; urea - 5.8 mmole/l.

December 2, 1996. Blood sugar - 3.9 mmole/l.

December 3, 1996. Urinanalysis: amount - 60.0 ml; straw color, transparent, acidic; sugar – neg; protein – neg; squamous epithelium – small amount; leukocytes, 2-3 in the field of microscope.

December 2, 1996: ECG: rhythm is sinus and regular. Position of the electrical axis of the heart is normal. Dystrophic changes of myocardium.

December 2, 1996: Examination by Neurologist. Findings: condition of the patient has substantially improved as compared to the previous examination in October 1996: he started performing self-service, i.e. dressing and eating unassisted. On examination: horizontal nystagmus at looking sideways still exists, rhinolalia has decreased. Muscular tension in arms is normal, in legs - high, s>d. Reflexes are high, s>d; Positive great-toe reflex on both sides. Coordination motor disturbances are not observed. Sensitivity is preserved.

Diagnosis: Multiple sclerosis, cerebrospinal form. Recommendations: continuation of cell therapy.

December 2, 1996 – administration of suspension No.2 (sample 3038AL118; cell count - $74 \cdot 10^6$/ml; CFU-GM - $32 \cdot 10^3$/ml; CFU bl. - $3.4 \cdot 10^3$/ml, and PC CD_{34} - $7.6 \cdot 10^3$/ml) intravenously, dropwise, in the amount of 4.0 ml; additional administration of suspension No.8 (sample 3038AM118; cell count - $89 \cdot 10^6$/ml), subcutaneous, in anterior abdominal wall, in two depots, in the amount of 1.0 ml each.

December 3, 1996, the patient noted a decrease in hypertension in the extremities, felt stronger. He slept well. He was working on computer all day long.

December 4, 1996 - administration of suspension No.2 (sample 3038P513Z; cell count - $63 \cdot 10^6$/ml; CFU-GM - $103 \cdot 10^3$/ml; CFU bl. - $3.2 \cdot 10^3$/ml, and PC CD_{34} - $7.2 \cdot 10^6$/ml) intravenously, dropwise, in the amount of 2.0 ml; additional administration of suspension No.8 (sample 3038P513M; cell count - $41 \cdot 10^6$/ml), subcutaneous, in anterior abdominal wall, in two depots in the amount of 1.5 ml each,.

At the time of discharge from the Clinic, the patient noted a decrease of hypertension in the extremities and neck muscles; he was feeling a burst of strength, and was in the mood for recovery and restoration of lost motions. Recommendations: physical exercise, including swimming pool training, and continuation of treatment in April 1997.

The patient was admitted to the Cell Therapy Clinic for the third time on April 23, 1997, and stayed there till April 29, 1997.

The patient notes a considerable improvement of his condition: he stands up and stands by himself, occasionally walks with a cane; his left foot twists at walking. Vision and hearing in the left ear have considerably improved. Bifurcation has disappeared, and there appeared a possibility of visual load, including work on personal computer. During previous trips, the patient was assisted by am accompanying person; this time, he is able to perform self-service (eating, dressing, toiletry and hygienic procedures etc.).

Neurological status: contactable, adequate, oriented.

Eye slits, pupils d=s, full range of motions of eyeballs, sufficient convergence; occasional, quickly disappearing, small-swinging horizontal nystagmus.

The face is symmetric, tongue in the midline. Range of active motions in arms is in full scope, muscular tension in arms is practically unchanged, reflexes are high, d≥s, with reflexogenous zone is expanded. Strength in legs is reduced, particularly in the right one, more in its distal parts. Tension is considerably increased, especially in flexors; more in the left leg, while in the right leg tension is combined due to the "cerebellum" hypotension. Reflexes are high, without any expansion of the reflexogenous zone; no foot clonus. Plantar reflex on the left, and an unconvincing great-toe reflex (Babinski's) on the right. Sensibility to pain is preserved. Finger-to-nose test is performed accurately. He stands leaning on a cane, and moves along the bed leaning on it.

Laboratory tests. Results of the total blood count from April 24 and 26, 1997 are given in Table 4.1, and immunology from April 24, 19976 - in Table 4.2.

April 26, 1997: Blood sugar - 4.3 mmole/l.

April 27, 1997: Blood Chemistry: total bilirubin - 15.1 mmole/l; direct – negative, indirect - 15.1 mmole/l; thymol test - 2.4 units; ALT - 0.38 mmole/l; AST - 0.21 mmole/l; beta lipoproteids - 47 units, creatinine - 0.074 mmole/l; urea - 6.9 mmole/l; total protein, 64 g/l,

including albumins - 52.1 %; and globulins - 47.4 % (including: α1 – 6.1 %; α2 – 9.9 %; β– 13.4 %, and γ – 18.0 %); seromucoid - 0.260 units; CRP - negative.

April 26, 1997: Urinanalysis: amount - 120.0 ml; straw color, transparent, acidic; relative density - 1020 g/l; sugar – neg; protein – neg; squamous epithelium – small amount; leukocytes – single, in the field of microscope.

April 28, 1997: Urinanalysis: amount - 60.0 ml; straw color, transparent, acidic; sugar – neg; protein – neg; squamous epithelium – small amount; leukocytes - 1-3 in the field of microscope.

April 26, 1997: ECG – rhythm is sinus and regular. Position of electrical axis of the heart is normal. Signs of moderate changes of myocardium.

April 24, 1997: Examination by Neurologist.

April 25, 1997: Ophthalmologist: preliminary test of *visus* in spectacles >0.6 OU. Refraction: hypermetropic astigmatism. Range of motions of eyeballs in full scope. Divergent squint OS (about 10 degrees on Girschberg scale). Small-swinging horizontal nystagmus at looking to the sides. Lively reaction of pupils to the light. Optical parts are transparent in passing light. Eyegrounds: disks of optical nerve are contoured, pale-pink. Arteries are narrowed. Arteries/veins=1/3. Delicate foci of dystrophy in macular areas OU. Diagnosis: Divergent concominant squint OS. Moderately manifested small-swinging nystagmus. Hypermetropic astigmatism. Angiopathy of retina. Initial maculodystrophy of both eyes.

Same diagnosis: multiple sclerosis, cerebrospinal form with tetraparesis, d>s, mainly manifested in legs. Divergent concominant squint OS. Moderately expressed small-swinging nystagmus. Hypermetropic astigmatism. Angiopathy of the retina. Initial maculodystrophy of both eyes.

January 24, 1997 – administration suspension No.2 (sample 3038P513Z; cell count - $63*10^6$/ml; CFU-GM - $103*10^3$/ml; CFU bl. - $3.2*10^3$/ml, and PC CD_{34} - $7.2*10^6$/ml), intravenously, dropwise, in the amount of 2.0 ml; additional administration of suspension No.8 (sample 3038P513M; cell count - $41*10^6$/ml), subcutaneous in anterior abdominal wall, in two depots, in the amount of 2.2 ml

January 27, 1997 – the same procedure: administered were 1.6 ml of the same suspension No.2 and 2.0 ml of the same suspension No.8.

The patient noted an increase of physical activity, growing easiness in movements, decrease of hypertension, improvement of the capacity for work, and improvement of mood.

Recommendations: to start exercising active movements of legs in the vertical position, using methods of mechanical therapy, and to continue treatment at the Cell Therapy Clinic.

The patient is in constant contact with Clinic's physicians. Observation is continued.

Example 5

Patient K., male, born in 1947, has been observed at the Clinic since February 1996.

On admittance to the Clinic on February 12, 1996, complained of constant headache, constant feeling of heaviness in the lower part of the abdomen, abdominal expansion and distention, general weakness, sweating, and lack of appetite. Speaks reluctantly, reserved. He believes that he has been offended by his fate. He is unhappy about his life. The patient has communication problems both with his family and colleagues. He does not cope with his job responsibilities.

History of present illness: constipation since early childhood. During his teenage period, he was diagnosed with dolichosigmoid, hypotonic dyskinesia of large intestine.

During last year, the patient was not able to defecate. Usual stimulators of the large intestine, such as saline, oily, and vegetable laxatives did not cause any effect, even in considerable amounts. Evacuation of the intestine was attained with saline-and-oily enemas, once in 3-4 and even 7 days. On examination: Skin is pale and ashy, facial expression was crestfallen, in low spirits.

Physical examination: blood pressure - 170/90; bradycardia - 52-54 beats/min; abdominal pain along large intestine, its segments were palpated as soft, dough-like expanded cylinders, their location usual. External hemorrhoid in the form of several painful, hyperemic, enlarged nodes.

Additional examination.

February 14, 1996: Irrigoscopy: barium sulphate suspension was filling loops of the large intestine very slowly. Dense filling was impossible. Large intestine is stretched by gas, its walls thinned. The lumen of the rectosigmoid section is considerably expanded, reaching 12 cm. Haustration is absent. Double contrasting revealed several additional spherical shadows of speckled structure located in the center of the intestinal lumen. During intestinal evacuation shadows of coproliths are shifted.

In 16 - 30 hours after per oral taking of barium sulphate suspension revealed was contrast along the whole large intestine and particularly in the proctosigmoid section.

Diagnosis: Megacolon; diffuse colostasis; coproliths.

Rectoromanoscopy (February 17, 1996). Depth of tubule insertion was 18 cm. Examination of the mucous coats of the large intestine is hindered by scybalous masses. The intestine is considerably expanded. Mucous coats are pale and atrophic, foldless and dull. Swollen and painful hemorrhoid nodes with signs of inflammation in the areas of internal and external sphincters.

Diagnosis: Megacolon; atrophy of the mucus; hypotonic-type dyskinesia; exacerbation of hemorrhoid.

Results of the total blood count and blood immunology are given in Tables 5.1 and 5.2

Final diagnosis: Megacolon; diffuse colostasis; exacerbation of chronic hemorrhoid; mild chronic anemia; systolic arterial hypertension; somatogenic depression.

February 20, 1996. – administration of suspension No.1 (sample PL-104; cell count – $62*10^6$/ml; CFU-GM - $53*10^3$/ml; CFU bl. - $2.3*10^3$/ml, and PC CD_{34} - $5.4*10^6$/ml), intravenously, dropwise, in the amount of 2.0 ml; administration of suspension No.8 (sample PM-104; cell count - $105*10^6$/ml), subcutaneous in anterior abdominal wall, in two depots, in the amount of 1.0 ml each. The procedure was tolerated well.

February 21, 1996. During examination noted is coloring of patient's cheeks. He was in elated mood, general weakness decreased, there was no headache; by the end of the day, there appeared inclination to defecation; defecation was without stimulation, in the form of doughy smears. Evacuation of intestine was not complete. Blood pressure - 120/180; pulse - 70 beats/min.

The patient was discharged on February 24, 1996, under observation of outpatient physician of the Clinic.

As it is stated in the outpatient card, at the end of February, in March and in April – several occasions of natural defecation of shaped scybalous masses. The patient started using cleansing enemas less frequently: once a week and even once in two weeks. He again switched to using saline, oily, and other known laxatives approximately once in 2 to 4 days in dosages increased by 50% from the accepted.

The patient became more active, he felt a burst of vital and physical strength; pain in the abdomen and heaviness in the lower part of the abdomen decreased, headaches bothered less frequently.

Examination: Blood pressure - 120-140/70-90.

May 14, 1996: Irrigoscopy, irrigography. Dense filling of intestinal loops with contrasting substance allows for detection of all intestinal sections. Haustration is preserved in blind gut, ascending and transverse colons, and is considerably less expressed in the sigmoid colon, which is elongated and expanded. Along colon-rectum passage, the lumen expands to 8 cm. Double contrasting revealed no additional formations against the ground of gas. In 16 hours

after per oral taking of barium sulphate suspension, contrast in the proctosigmoid section is preserved.

Diagnosis: Dolichocolon; hypotonic dyskinesia of the intestine.

May 17, 1996: Rectoromanoscopy: Depth of the tubule insertion was 22 cm. Intestinal lumen is expanded. Its mucous coats were pink and bright. Folding is smoothed. Internal and external hemorrhoid nodes are shrunk, of natural color, and without any signs of inflammation.

Diagnosis: Hypotonic-type dyskinesia of the intestine; expansion of ampulla recti; hemorrhoid in remission.

Results of peripheral blood count and blood immunology are given in Tables 1 and 2.

In September-December, 1996 the patient noted defecation without stimulation, once in 2 to 3 days; there appeared the possibility of its regulation by means of a diet and ballast substances of vegetable origin. Facial skin acquired natural color; the patient is still active; weakness is absent; appetite is preserved; headaches only in case of weather changes.

In March 1997, the patient contacted the Clinic in connection with worsening of general condition and relapses of steady constipation. He again started using cleansing enemas twice a week; there appeared resistance to laxatives.

March 13, 1997. – repeated administration of suspension No.1 (sample PL-104; cell count - $62*10^6$/ml; CFU-GM - $53*10^3$/ml; CFU bl. - $2.3*10^3$/ml, and PC CD_{34} - $5.4*10^6$/ml), intravenously, dropwise, in the amount of 2.0 ml; administration of suspension No.8 (sample PM-104; cell count - $105*10^6$/ml),subcutaneous, in anterior abdominal wall, in two depots, in the amount of 1.0 ml each. The procedure was tolerated well.

By the end of the month, patient's condition has substantially improved. At present, he feels well, maintaining his health problems and evacuation of the intestine on his own.

Example 6

Patient F., female, born in 1962, has been observed at the Clinic since May 10, 1995 in connection with neuro-circulatory dystonia, astheno-neurotic syndrome, and irritated bowel syndrome (*colon irritabile*).

The patient suffered from general weakness, worsening towards evening; headache; vertigo; permanent feeling of weariness; reduction of ability to work, disorders of sleep (during 6 months, the patient could fall asleep only after intake of soporifics); problems with appetite; extensive (diffuse) abdominal pain, sometimes violent, forcing the patient to moan, to support her abdomen in her hands, and to go to bed. Meteorism. Frequent diarrhea secondary to the slightest dietary changes. Stool disturbances were followed, for 2 - 3 days, by constipation accompanied by headache and spasmodic abdominal pain. Occasional abundant mucus in the stool.

On examination: Height - 168 cm. Weight - 52 kg. Narrow shoulders. Long extremities. Loose breasts and abdomen. Muscular system is not developed. Skin is pale and clear. Lymphatic nodes are not enlarged. Blood pressure - 100/70; pulse - 88 beats/min, arhythmic; extrasystolic. Respiration rate - 18/min. Heart sounds are clear, binomial melody; auscultation reveals systolic murmur at the *apex cordis*. Clear percussion sound above lungs. Lung fields are clear, breath is weakened. At palpation, abdomen is soft and tender along large intestine. Rumbling in blind gut. Greater curvature of the stomach is palpated 2 cm below umbilicus, in the midline. Sigmoid colon is palpated as a painful segment in the suprapubic region. No visible edemata.

Results of additional examinations.

May 12, 1995: Rectoromanoscopy: Rectal spasm didn't allow for tubule insertion deeper than 20 cm. This process was problematic. Rectum could be extended only by air flow. Mucous coats were pale, bright, covered with mucus; several zones of hyperemia. Folding was usual.

Diagnosis: Irritated bowel syndrome. Hyperkinetic-type dyskinesia.

May 14, 1995: Irrigoscopy, irrigography. Contrasting enema allows for detection of all sections of the large intestine in slow retrograde manner. All intestinal sections are lowered by 5-10 cm in relation to the reference points of skeleton. Haustration is strong and irregular; it is better manifested in sigmoid ascending and descending colons. The pattern of mucous coats is variegated, folds are widened, pillow-like, their number reduced. Alternation of spasmodic and relaxed segments is observed. Acute spasmodic stenosis in the area of the spleen curvature. Ampulla recti is decreased in size and spastic. There were no filling defects. Evacuation is inhibited. In 16 hours after per oral intake of contrasting substance, revealed are contrasting of feces along the whole length of the intestine, other aspects – as described above.

Diagnosis: Coloptosia . Syndrome of irritated large intestine.

May 15, 1995. - administration of suspension No.1 (sample F-58; cell count - $72*10^6$/ml; CFU-GM - $164*10^3$/ml; CFU bl. - $5.6*10^3$/ml, and PC CD_{34} - $3.2*10^6$/ml), intravenously, dropwise, in the amount of 1.0; administration of suspension No.8 (sample F-58M; cell count - $72*10^6$/ml), subcutaneous, in anterior abdominal wall, in two depots, in the amount of 1.0 ml each.

The procedure was tolerated well. By the evening of the same day, she felt better, abdominal pain decreased, headache disappeared; the patient became more active. She could not fall asleep. Prescription of a soporific was necessary. Next morning she experienced psychoemotional animation and felt better. Appetite has improved; she spent the day in a more active way. Pain syndrome disappeared. In the evening, she had stool without stimulation, that was somewhat stiff, with mucus. Later on, she slept without any soporific. The patient was discharged from the Clinic on May 18, 1995 with improved general condition.

July 17, 1995: Control rectoromanoscopy. Tubule insertion was easy, depth – 26 cm. Mucous coats were clean, smooth, pink, and bright. Folding was usual.

Conclusion: no functional or morphologic pathology.

July 19, 1995: Irrigoscopy, irrigography. Retrograde insertion of barium sulphate allows for detection of all sections of the large intestine, that somewhat lowered. Haustration is regular, observed in all sections. Evacuation is timely. The pattern of mucous coats is delicate and manifested in all sections. No filling defects or deformations. Double contrasting (1000 ml of air) revealed elastic intestinal walls, and intestinal lumen is not changed. Additional formations are not detected against the ground of gas.

Conclusion: no functional or organic pathology of large intestine was detected.

In August, staying at a health resort, the patient suffered from alimentary toxoinfection caused by consumption of a poor-quality food (cream dessert). She was taking an antibiotic. Diffuse abdominal pain and stool disturbance relapsed, production of mucus was increased. The patient contacted the Clinic on August 30, 1995. After examination, rectoromanoscopy, and bacteriological study of feces, diagnosis was the same: *colon irritabile*.

September 2, 1995 – administration of the same suspension No.8 was administered, procedure and amount being the same. The procedure was tolerated well. During the first week pain attacks were cupped of, psychoemotional condition improved, motor activity increased, there appeared regular and shaped stools. Defecation was bringing satisfaction.

The patient contacted the Cell Therapy Clinic once again in March, 1996, after a psychological trauma (her mother's death). The clinical picture of disease came back: stable diarrhea; stool was liquid, abundant, and foamy. Abdominal distention and gas discharge was intensified. Smell of feces and gases changed. It was similar to yeast ferment. The patient noted worsening of her condition after eating carbohydrate-containing food, such as bread, groats porridge, fruit, and sweets. There appeared irritation and painfulness in the anal area.

March 29, 1996: Rectoromanoscopy: Depth of tubule insertion was 20 cm. Rectum was spasmodic. Its mucous coats were hyperemic, in a spot-like pattern, covered with foamy white mucus; numerous dot-like erosions on the rear surface of ampulla recti.

Diagnosis: Catarrhal, erosive proctosigmoiditis. Fermentative dyspepsia. Inoculation of intestinal flora was recommended.

March 29, 1996: Bacteriological study of feces. *Candida albicans* were detected. Amount of bifidobacteria was reduced down to 10^6/g, normal value being 10^8-10^{10}/g feces. Results of peripheral blood count and blood immunology are given in Tables 6.1 and 6.2.

Diagnosis: Erosive proctosigmoiditis. Hyperkinetic-type dyskinesia of large intestine. Intestinal dysbacteriosis. Fermentative dyspepsia.

Prescriptions: *Nistatini*, doses of 500,000 units six times a day for 10 days; *Bifidumbacterini Sicci*, triple dosages 3 times a day, for 28 days, and diet with reduced amount of carbohydrates.

April 5, 1996 - administration of suspension No.7 (sample FG-58; cell count - $45*10^5$/ml), subcutaneous, in the right and left external quadrants of buttocks, in two depots, in the amount of 1.5 ml each; administration of suspension No.8 (sample FM-58, cell count - $72*10^6$/ml) in anterior abdominal wall.

The procedure was tolerated well. On the next day, she felt much better. Frequency of stools decreased, abdominal pain disappeared. At night she was sleeping quietly.

Within one month, abdominal distention and pain, as well as painfulness in the anal area, have disappeared. Stool was once a day. Feces acquired shape and usual smell.

Rectoromanoscopy performed on April 10, 1996, did not reveal any organic or functional pathology of rectosigmoid section of the large intestine. Examined mucous coats were clean, smooth, bright, of natural color. During examination there were no rectal spasms..

According to the patient, her condition remained stable till the end of April, 1997, when she endured acute adenovirus respiratory infection. Diffuse pain in abdomen appeared, it was associated with eating and defecation. Cases of unstable stool reappeared. In May 1997, the patient had gynecological surgery (abortion, 12 weeks). Postoperative period proceeded with complications. The patient was treated for endometritis. She received several courses of antibiotics, which resulted in several cases of uterine hemorrhages.

June 5, 1996: Diagnostic and medical curettage of the uterus cavity. Within a month after this procedure, clinical symptoms of reproductive organs started to disappear. Leucorrhea disappeared; pain in the lower part of the abdomen stopped, menstrual cycle went right; at the same time, general weakness and headache sharply increased; vertigo, palpitation, and diffuse pain in abdomen appeared, relapsed production foamy mucus. Stools became frequent and liquid again.

Examination: rectoromanoscopy, bacteriological study of feces, general blood test and immunological blood test.

Results:

July 8, 1997: Rectoromanoscopy: syndrome of irritated intestine.

July 8, 1997: Bacteriological study of feces: *Candida*-type yeast fungi. Amount of bifidobacteria was reduced down to 10^7/g feces.

July 8, 1997: Total blood count : medium-degree anemia (see Table 6.1).

Immune status: Reduced low - T-lymphocytes and T-helpers; high - B-lymphocytes and G-immunoglobulins.

Prescriptions: *Nistatini*, 500,000 units 6 times a day for 10 days; diet with reduced amount of carbohydrates.

July 15, 1995 – administration of suspension No.1 (sample F-58; cell count - $72*10^6$/ml; CFU-GM - $164*10^3$/ml; CFU bl. - $5.6*10^6$/ml, and PC CD_{34} - $3.2*10^6$/ml), intravenously, dropwise, in the amount of 2.0 ml; administration of suspension No.8 (sample F-58M; cell count - $72*10^6$/ml), subcutaneous, in anterior abdominal wall, in two depots, in the amount of 0.5 ml each.

The procedure was tolerated well.

On the next day, July 16, 1997, she felt better, and noted decreased weakness, headache, and vertigo. Her appetite has improved. Her cheeks acquired coloring. Abdominal pain and gas formation have decreased. Stools frequency was substantially decreased.

By the end of the first weak, blood parameters were normalized, and in 14 days noted was an increase and normalization of immune indices. Within one month, clinical symptoms associated with *colon irritabile* were gone. Abdominal pain has disappeared. Gas formation has substantially decreased, abdominal distention stopped. Stools frequency returned to regular (once a day); feces acquired usual consistence and shape. Mucus formation has substantially decreased. Headache and vertigo stopped. The patient did not feel any fatigue during the day. She gained 4 kg. Till present moment, the patient has not been complaining of any health problems. She is active and cheerful, goes in for sport.

Example 7

Patient O., female, born in 1964, has been observed at the Cell Therapy Clinic since January, 1996. Considers herself sick since January, 1995 when abdominal stools became more frequent (up to 8 times a day), with admixtures of mucus and blood. These symptoms vanished by themselves. In October, stools gradually became more frequent (up to 12 times a day), with admixtures of mucus and blood. During several weeks, body temperature rose to 38° C; the patient lost 5 kg in weight; she noted constant weakness, sweating, reduction of ability to work. In November 1995, she was examined at the Infection and Therapeutic Departments. Bacteriological study of feces and serologic blood tests allowed to exclude infectious nature of the disease.

Performed were the following procedures: total blood count (Table 7.1), blood immunology (Table 7.2), colonoscopy (November 12, 1995), and irrigoscopy (November 14, 1995).

Colonoscopy findings: colonoscope was inserted into the caecum cupola and through the ileocecal valve into the small intestine, depth of insertion was 10 cm. Mucous coats of small intestine were pale-pink and velvety. The ileocecal valve was crescent-shaped and oriented towards the lumen of the caecum cupola. Mucous coats of caecum, ascending colon, and transverse colon were unremarkable. Mucous coats of descending colon, sigmoid colon, and rectum were hyperemic, friable, swollen, infiltrated, with fibrin deposits, numerous erosions, separately located longitudinal ulcers, and single inflammatory polyps. Also detected were segmental regions of intact mucous coats with some ulcerative defects. Vascular pattern was blurred, haustration smoothed. Moderate amount of sanguineopurulent discharge was detected in the intestine lumen. Samples were taken from 3 various regions of the intestine.

Diagnosis: Crohn's disease of the left half of large intestine.

Biopsy results: examined were 6 byopsy samples of mucosa and submucous layer of the large intestine. Expressed leukocyte infiltration of mucous coats submucous layer by mononuclears. Fine hyalinized vessels; regions of fibrosis in submucous layer, elements of granuloma with multinuclear gigantic cells.

Diagnosis: Granulomatous colitis (Crohn's disease).

Irrigoscopy findings: administration of contrasting suspension reveals expressed unclear and irregular contours along the length from the rectum and to the medium third of the transverse colon. The lumen is irregularly narrowed, gaustra are straightened, left sections of intestine are shortened. Numerous erosions, ulcers, small and big defects both along contours and in the lumen. Numerous spicule-like diverticula in the sigmoid section of the intestine. Transverse colon is sagging, the caecum cupola is situated is lowered. Evacuation is complete; the pattern of the mucous coat in right sections – folds are preserved. Vertical examination of the patient revealed ptosis of all the sections of the large intestine.

Diagnosis: Crohn's disease, predominantly left-side lesion.

The patient was prescribed 4g of Salofalk daily, *per os*; infusion therapy was carried out with isotonic solution of sodium chloride, glucose solution, and neohemodez. In November and December of 1995, frequency of stools decreased to 4 times a day; amount of admixtures in feces was reduced; abdominal pain became less acute. After some excessive meals and physical load during Christmas vacations of 1996, and secondary to intake of 4 g of Salofalk daily, frequency of stools again increased up to 8-12 times a day; abdominal pain became more acute, blood admixtures appeared in feces.

The patient was admitted to Cell Therapy Clinic on January 18, 1996. On examination: skin is pale, the patient is exhausted, lost 8-10 kg. Skin turgor is reduced. The patient notes constant weakness and sweating during the day. She cannot work. Body temperature - 37.3 °C; pulse - 98 beats/min, rhythmic, blood pressure - 90/60. Heart sounds are clear and rhythmic. Lung fields are clear. Abdomen is soft and asymmetric; slight diverticulum of the left half of abdomen.

Diagnosis: Crohn's disease, predominantly left-side section. Acute phase. Light-degree anemia.

Results of the total blood count and blood immunology are given in Tables 7.1 and 7.2.

January 22, 1996, - administration of suspension No.1 (sample S-673; cell count – $110*10^6$/ml; CFU-GM - $58*10^3$/ml; CFU bl. - $1.2*10^3$/ml, and PC CD_{34} - $2.4*10^6$/ml), intravenously, dropwise, in the amount of 1.9 ml; administration of suspension No.7 (sample S-673E; cell count - $81*10^5$/ml) in the amount of1.0 ml.

During first days upon administration, the patient felt better, more cheerful. Her mood changed, she began to believe in recovery. The time spent out of the bed increased. She began consuming more food. On the first day after administration, stools frequency was 6 times; on the next day - 5 times, on the 3rd, 4th, and 5th days - 4 times. Visible admixtures of blood in feces disappeared by the 7th day. Gregersen's reaction (presence of occult blood in feces) was positive for 2 more months, later on it became stably negative. During the 2-month period, the patient gained 5 kg; stools frequency reduced to 1-3 times a day, without any admixtures of mucus and blood. Pain in the left side of abdomen was decreasing during 14 days and then disappeared.

At the end of February, control tests were performed. Results of peripheral blood count and blood immunology are given in Tables 7.1 and 7.2, respectively.

February 26, 1996: Colonoscopy findings: examined were the whole large intestine and 10 cm of small intestine. Mucous coats of the small intestine were without any changes. Mucous coats of caecum and ascending colon were succulent and loose; vascular pattern is blurred; folds are high, dense, and scalloped. Mucous coats of the transverse colon are pearl-white, vascular pattern is clear. Omental tenia is clearly visible. Mucous coats of the descending colon are smooth, bright, light-pink; vascular pattern is clearly displayed. Mucous coats of sigmoid colon and rectum are bright red, clean, with individual erosions on fold ridges; vascular pattern is blurred; large submucous vessels are well distinguishable. Haustration is reduced.

Diagnosis: Crohn's disease of the left half of large intestine. Remission.

Irrigoscopy findings: Retrograde application of contrasting enema allowed for detection of all sections of the large intestine. Haustration is regular and somewhat smoothed in the rectum and sigmoid colon. Rigidity of walls and irregularity of sigmoid colon contour remain. Stable barium depots are present on its relief. Pattern of the mucous coats is delicate, no spicules are observed. Evacuation is accelerated.

Double contrasting (1000 ml of air) revealed no additional formations against the ground of gas.

Diagnosis: Crohn's disease of the large intestine, rectosigmoid section, beginning exacerbation.

Patient's condition was satisfactory till October 1996 when abdominal pain reappeared and stools became more frequent, up to 3-4 times a day. No pathologic admixtures of blood and mucus were present in feces; however, Gregersen's reaction became stably positive again. On

October 16, 1996, rectoromanoscopy was performed: mucous coats of rectum and sigmoid colon were hyperemic, loose and edematic, with numerous erosions. Vascular pattern was blurred. Contact with instruments resulted in bleeding of mucous coats.

Diagnosis: Erosive proctosigmoiditis. Crohn's disease, beginning exacerbation. Total blood count and immunologic blood test were performed. Since there were no irregularities observed, suspensions Nos 1, 2, 3, and 4 were not administered.

October 18, 1996 - administration of suspension No.7 (sample S-673E; cell count - $14*10^5$/ml), subcutaneous, in anterior abdominal wall, in two depots in the amount of 2.0 ml- 1.0 ml each; administration of suspension No.5 (sample S-673H; cell count - $2.7*10^6$/ml) subcutaneous, in external quadrants of buttocks, in two depots, in equal amounts, totaling to 3.0 ml.

The patient noted a decrease in weakness and sweating; abdominal pain disappeared within a week. 8 days later, Gregersen's reaction became negative. Stools frequency of 1-2-3 times a day restored during one month. Repeated rectoromanoscopy performed on November 20, 1996 revealed no signs of erosive proctosigmoiditis.

Rectoromanoscopy findings: Depth of tubule insertion was 22 cm. Intestinal mucous coast were clean, smooth, and bright red in some loci. No erosions or ulcers.

Diagnosis: Crohn's disease, remission.

Observation is continued.

Example 8

Patient A., male, born in 1938, has been observed at the Cell Therapy Clinic since March, 1994. According to the patient, the disease started in December, 1993 when there appeared fatigue, reduced capacity for work, lack of appetite, and skin paleness.

He was treated by General Physician and consulted by Hematologist and diagnosed with light-degree iron deficiency anemia. The patient received iron preparations, and followed dietary therapy. Hemoglobin increased from 110 g/l to 130 g/l. In February 1994, red blood count decreased again; he lost 4 kg; fatigue came back. The patient was examined at the Therapeutic Department where he was diagnosed with rectum cancer (in the superampullar section, saucer-shaped cancer of adenocarcinoma type).

Diagnosis: rectum cancer T3N3MOG1 (stage C2 by Duke). The patient was transferred to the surgical department for surgical treatment. At that time, he lost 7 kg; he suffered from acute weakness, vertigo, sweating, diarrhea with streaks of scarlet blood, absence of appetite, and fevers up to 38 °C, temperature rose by the evening time. The patient was spending almost all day in bed. Laboratory tests revealed the following irregularities: erythrocytes - 2.4 T/l; hemoglobin - 76 g/l; leukocytes - 3.8 g/l; erythrocyte sedimentation rate (ESR) - 44 mm/hr; totlal protein - 62 g/l; albumins - 45 %; globulins - 55 % (including: α1– 6 %; α2– 14 %; β– 13 %, and γ-globulin – 22 %).

During pre-operational stage, neither blood restoring preparaitons nor transfusions of erythrocytes were effective in increasing of erythrocyte count and hemoglobin. Prescribed antibiotics and detoxication therapy failed to lower his body temperature. Intoxication phenomena were building up; the patient lost much weight.

March 5, 1994 - administration of suspension No.1 (sample FL-406; cell count - $170*10^6$/ml; CFU-GM - $88*10^3$/ml; CFU bl. - $2.3*10^3$/ml, and PC CD_{34} - $4*10^6$/ml), intravenously, dropwise, in the amount of 2.0 ml.

On March 6, the patient noted a decrease in weakness and sweating; temperature was normalized; appetite has improved. He could get up from the bed, and spent several hours in an armchair.

March 8, 1996: Total blood count: erythrocytes - 2.2 T/l; hemoglobin - 70 g/l; leukocytes - 5.2 g/l; ESR - 16 mm/hr.

With every day, the patient was feeling better. On March 14, erythrocyte count was 3.8 T/l; hemoglobin, - 132 g/l; leukocytes - 5.6 g/l; ESR - 22 mm/hr. He became more active, did not feel weak during daytime; stools restored to 1-2 times a day, still with streaks of blood but in the form shaped scybalous masses.

Body temperature was normalized; several times temperature rose up to 37.3°C during evening hours; the patient gained 2 kg.

March 16, 1994: Radical surgery: Colectomy with ileoanal endorectal anastomosis.

Final diagnosis: Rectum cancer, stage C by Duke, T3N1MOG1.

Postoperative period proceeded satisfactory. The patient was discharged from the Clinic on March 28, 1994. Recommendations: chemotherapy starting April 1 at the Chemotherapy department of the Research Institute for Oncology.

The following chemical preparations were prescribed for 3 months: Vincristin - 1 mg daily, intravenously, once in 3 weeks; 5-ftoruracyl, intravenously, for 4 hours, from the 1st till 7th day of every 3 weeks.

This treatment was tolerated well till May 9 when suddenly he felt pain in the mouth and throat, temperature rose to 38.7 °C; otic and submandibular lymphatic nodes enlarged and were tender at palpaion. Examination of the oral cavity and posterior wall of the throat revealed hyperemia of mucous coats and numerous erosions; a crater-shaped ulcer with dark edges was found on the right tonsil.

Total blood count: erythrocytes - 3.2 T/l; hemoglobin - 90 g/l; leukocytes - 0.8 g/l; thrombocytes - 170 g/l; ESR - 55 mm/hr. Proteinuria and erythrocyturia. Diagnosis was: Agranulocytosis; ulceronecrotic angina; erosive mucositis.

May 11, 1994 - administration of suspension No.1 (sample F-406; cell count - $170*10^6$/ml; CFU-GM - $88*10^3$/ml; CFU bl. - 2.310^3/ml, and PC CD_{34} - $4\cdot10^6$/ml), intravenously, dropwise, in the amount of 1.0 ml

By the evening of the same day, body temperature fell to 37.5 °C; the patient noted a decrease of weakness.

In the morning of May 12, he was cheerful; pain in the oral cavity decreased; hyperemia of mucous coats acquired cyanotic tint; a fibrinous scab appeared on the right tonsil. No new erosions. Lymphatic nodes were enlarged but indolent; body temperature during the day was sub febrile. Data on Total blood count from May 12, 1994 is given in Table 8.1. By May 19, blood indices were completely restored (see Table 8.1).

The patient continued chemotherapy, and during 1994, he received 6 courses without any complications from the side of blood and internal organs.

December 19, 1994 - with the aim of correcting blood indices- administration of suspension No.2 (sample F-406L; cell count - $93*10^6$/ml; CFU-GM - $54*10^3$/ml; CFU bl. - $4.8*10^3$/ml, and PC CD_{34} - $9*10^6$/ml), intravenously, dropwise, in the amount of 1.0 ml; administration of suspension No.6 (sample F-406T; cell count - $109*10^4$/ml), in the amount of 3.0 ml

Initial and restored indices of immune status are given in Table 8.2.

The patient was observed at the Department of Oncologic Surgery. Follow-up examinations were performed once in every six months.

Procedures performed included total blood count, immunology; rectoromanoscopy; sonoradiography liver and abdominal organs; irrigoscopy (once a year); and colonoscopy (once a year). Patient's condition was satisfactory.

In 1997, detected in the anastomosis area were 2 polyps sizing 5 and 7 mm, caused by inflammation (results of biopsy carried out on March 12). Also noted was a decrease of total lymphocyte count, decrease of the indices of T-lymphocytes, helpers, and NK-cells.

March 14. 1997 - intravenous administration of the same suspensions No.2 in the amount of 1.0 ml and suspension No.6 in the amount of 1.5 ml.

Presented are results of immunological and total blood counts, performed on April 16 and September 12, 1997. Rectoromanoscopy from April 16, 1997 revealed no polyps on the rectal mucous coats in the anastomosis area. Observation is continued.

Industrial Applicability

As can be seen from the above Examples, cell therapy of the invention allows for rather successful achievement of expressed and fairly stable curative effect in those diseases whose treatment by conventional methods was impossible.

The above-mentioned results can be explained by systemic impact of cell suspensions on patients, manifested :

first, in replenishing of the recipient's body with histocompatible "building material" in the form of multitude of non-specialized or low-specialized embryonic cells that are distributed by the blood flow to organs and tissues and, by forming new cell associations, proliferate and restore lost or substantially weakened functions;

second, in producing - by the above-described cells – of biologically active substances that are distributed by blood and lymph flows throughout the recipient's body and exert a medicine-substituting effect.

Indeed, the clones of specialized cells, formed from the material of administered to the patients cell suspensions, are capable of long-term *in situ* synthesis of the substances that patient would have otherwise taken as medicinal preparation.

Appendix

EFFECTIVENESS OF CELL THERAPY OF THE INVENTION

Introductory comments:

a) in Table numbers, the first digit corresponds to Example No., and the second digit identifies the Table number "inside" a relevant Example;

b) abbreviation "CT" means "cell therapy of the invention";

c) normative values of indices are given according to the patients' sex.

Table 2.1

Peripheral Blood Count After First Administration

Parameters	Prior to CT	After CT (day)						
		1st	2nd	7th	11th	15th	25th	41st
Erythrocytes, 10^{12}/l	1.3	1.8	2.2	2.2	2.5	2.1	1.4	1.6
Hemoglobin, g/l	61	60	72	74	82	74	60	62
Color index	0.9	0.9		1.0	1.0	1.0		1.1
Leukocytes, 10^9/l	2.4	2.0	1.5	2.6	1.6	2.4	2.1	2.3
Basophils								1
Eosinophils								4
Metamyelocytes				2				
Stab cells (neutrophiles)	3	1		6	1	4		4
Segmenticulars	17	15		24	32	32		33
Lymphocytes	74	78		62	61	56		50
Monocytes	6	6		6	6	8		6
Thrombocytes, 10^9/l								
ESR, mm/hr	36	21		66	64	32		43
Mononuclears								Normoblast 4:100

Table 2.1 (continued)

Peripheral Blood Count After Second Administration

Parameters	3rd day	7th day	30th day	48th day	2 months	2.5 months	11 months
Erythrocytes, 10^{12}/l	1.4	1.6	2.4	2.5	3.2	4.2	4.8
Hemoglobin, g/l	58	54	75	88	136	142	148
Color index	1.0						
Leukocytes, 10^9/l	2.4	2.2	3.0	4.2	3.5	3.7	4.6
Basophils	1	1		1	1	1	1
Eosinophils	4	2		4	2	1	3
Metamyelocytes							
Stab neutrophils	4			26	2	4	6
Segmenticulars	19				35	21	35
Lymphocytes	59			61	54	65	52
Monocytes	15			8	6	8	3
Thrombocytes, 10^9/l	32	84	103	118	220	220	215
ESR, mm/hr	40	44	50	18	6	4	7
Mononuclears			3				

Table 2.2

Absolute Contamination of Bone Marrow-Forming Elements

Parameters	Prior to CT	9th day after 1st CT, 10^9/l	15th day after 2nd CT, 10^9/l	24th day after 2nd CT, 10^9/l
Neutrophilocytes, promyelocytes	0.01	0.02	0.1	16.28
Neutrophilocytes, myelocytes	0.06	0.86	0.1	32.5
Neutrophilocytes, metamyelocytes	0.01	0.75	0.6	28.86
Neutrophilocytes, stab cells	0.09	0.33	0.86	27.48
Neutrophilocytes, segmenticulars		0.05	0.63	17.5
Eosinophils, segmenticulars	0.01	0.1	0.1	6.28
Basophils, segmenticulars	0.11	0.46	0.3	1.18
Erythroblasts	0.05	0.86	0.5	12.04
Normocytes, basophilic	0.138	1.13	0.8	22.00
Normocytes, polychromatophilic	0.07	2.33	2.3	43.36
Normocytes, oxyphilic	0.014	0.06	0.16	12.16
Lymphoblasts	0.01	0.33	0.03	7.16
Lymphocytes	1.33	2.43	2.06	23.64
Holonuclears	0.1	0.06	0.26	6.32
Plasma cells	0.01	0.23	0.3	0.34
Megakaryocytes "in preparation" (in the field of microscope)	0	Isolated	2:25 do not function	2:10000 function
Megakaryocytes, 10^9/l	2	10	10	180

Table 2.3

Immunology

Parameters	Norm	12.17.1994	12.28.1994	02.03.1995	07.06.1996
Lymphocytes,abs. in 1 μl	720-3600	1012	2128	1408	1316
T-lymphocytes (CD_3)	701-2005	314	1274	760	763
CD_3, %	55-83	31	60	54	58
CD_4, %	27-56	18	29	31	35
T-suppressors (CD_8)	146-810	128	518	394	290
CD_8, %	15-37	12.6	24.3	28	22
CD_4/CD_8 ratio	0.6-2.8	1.42	1.19	1.10	1.59
NK-cells, $CD_{16}+CD_{56}$	70-350	91	211	280	157
$CD_{16}+CD_{56}$, %	4-18	9	10	13	12
B-lymphocytes (CD_{19})	22-530	66	314	225	197
CD_{19}, %	4-24	65	14.7	16	15
B-2 microglobulins	0.8-2.6	0.9	2.4	2.8	2.2
Immunoglobulins:					
A, g/l	1.03-4.04	0.7	3.0	4.6	3.7
G, g/l	6.64-14.0	3.2	12.4	16.4	12.6
M, g/l	0.55-1.41	0.3	1.3	1.2	1.6

Table 2.3 (continued)

Parameters	Norm	03.11.1997	04.10.1997	09.18.1997
Lymphocytes abs. in 1 μl	720-3600	984	1598	1350
T-lymphocytes (CD_3)	701-2005	423	815	567
CD_3, %	55-83	43	51	42
CD_4, %	27-56	41	38	39
T-suppressors (CD_8)	146-810	344	431	351
CD_8, %	15-37	35	27	26
CD_4/CD_8 ratio	0.6-2.8	1.17	1.41	1.50
NK-cells, $CD_{16}+CD_{56}$	70-350	138	176	243
$CD_{16}+CD_{56}$, %	4-18	14	11	18
B-lymphocytes (CD_{19})	22-530	197	288	203
CD_{19}, %	4-24	20	18	15
B-2 microglobulins	0.8-2.6	2.1	1.9	2.3
Immunoglobulins:				
A, g/l	1.03-4.04	3.2	4.1	4.2
G, g/l	6.64-14.0	18.1	12.0	11.0
M, g/l	0.55-1.41	1.2	0.8	0.8

Table 3.1

Peripheral Blood Count

Parameters	Norm	05.16.1996	07.01.1996
Erythrocytes, T/l	4.0-5.5	4.2	4.2
Hemoglobin, g/l	132-165	130	136
Color index	0.82-1.05	0.9	0.9
Thrombocytes, 10^9/l	180-320	180	200
Leukocytes, g/l	4-8.8	4.0	3.9
Eosinophils	≤6 %	3	2
Stab neutrophils	≤6 %	2	2
Segmenticulars	≤70 %	71	59
Lymphocytes	20-40 %	20	32
Monocytes	2-9 %	4	5
ESR	1-10	3	3

Table 3.2

Immunology

Parameters	Norm	05.16.1996	07.01.1996
Lymphocytes abs. in 1 μl	720-3600	800	1248
T-lymphocytes (CD_3)	701-2005	272	636
CD_3, %	55-83	34	51
T-helpers (CD_4)	357-1254	144	537
CD_4, %	27-56	18	43
T-suppressors (CD_8)	146-810	88	349
CD_8, %	15-37	11	28
CD_4/CD_8 ratio	0.6-2.8	1.64	1.53
NK-cells, $CD_{16}+CD_{56}$	70-350	120	237
$CD_{16}+CD_{56}$, %	4-18	15	19
B-lymphocytes (CD_{19})	22-530	128	150
CD_{19}, %	4-24	16	12
B-2 microglobulin	0.8-2.6	3.2	2.2
Immunoglobulins:			
A, g/l	1.03-4.04	1.4	1.2
G, g/l	6.64-14.0	20.2	14.3
M, g/l	0.55-1.41	0.9	0.7

Table 4.1

Peripheral Blood Count

Parameters	Norm	10.07.1996	10.08.1996	10.10.1996	12.02.1996
Erythrocytes, T/l	4.0-5.5	4.95	4.8	4.0	4.6
Hemoglobin, g/l	132-165	160	160	130	152
Color index	0.82-1.05	1.0	1.0	0.9	1.0
Thrombocytes	180-320	260	290	300	210
Leukocytes, g/l	4-8.8	7.4	5.6	5.0	6.8
Eosinophils	≤6 %	1	3	3	2
Stab neutrophils	≤6 %	3	4	8	4
Segmenticulars	≤70 %	68	69	65	54
Lymphocytes	20-40 %	26	21	21	39
Monocytes	2-9 %	2	3	3	1
ESR	1-10	3	2	2	3

Table 4.1 (continued)

Peripheral Blood Count

Parameters	Norm	12.04.1996	04.24.1997	04.26.1997
Erythrocytes, T/l	4.0-5.5	4.5	4.4	4.3
Hemoglobin, g/l	132-165	150	146	146
Color index	0.82-1.05	1.0	1.0	1.0
Thrombocytes	180-320	270	190	200
Leukocytes, g/l	4-8.8	6.0	5.2	8.0
Eosinophils	≤6 %	2	2	2
Stab neutrophils	≤6 %	3	3	1
Segmenticulars	≤70 %	64	65	60
Lymphocytes	20-40 %	28	22	31
Monocytes	2-9 %	3	8	7
ESR	1-10	5	3	3

Table 4.2

Immunology

Parameters	Norm	10.07.1996	12.02.1996	04.26.1997
Lymphocytes abs. in 1 μl	720-3600	1924	2652	2430
T-lymphocytes (CD_3)	701-2005	827	1538	1743
CD_3, %	55-83	43	58	72
T-helpers (CD_4)	357-1254	693	1113	941
CD_4, %	27-56	36	42	54
T-suppressors (CD_8)	146-810	847	822	850
CD_8, %	15-37	44	31	35
CD_4/CD_8 ratio	0.6-2.8	0.82	1.35	1.54
NK-cells, $CD_{16}+CD_{56}$	70-350	135	318	194
$CD_{16}+CD_{56}$, %	4-18	7	12	8
B-lymphocytes (CD_{19})	22-530	500	398	413
CD_{19}, %	4-24	26	15	17
B-2 microglobulins	0.8-2.6	3.1	1.9	1.6
Immunoglobulins:				
A, g/l	1.03-4.04	3.6	3.2	3.9
G, g/l	6.64-14.0	18.4	12.5	14.3
M, g/l	0.55-1.41	0.8	0.9	0.74

Table 5.1

Peripheral Blood Count

Parameters	Norm	Prior to CT	After CT (day)			
			3rd	14th	160th	150th
Erythrocytes, T/l	4.0-5.5	3.4	3.2	4.2	4.3	4.5
Hemoglobin, g/l	132-165	110	105	118	120	125
Color index	0.82-1.05	0.8	0.8	0.9	0.9	0.9
Reticulocytes, %	0.2-1.2	0.6	0.8	1.2	0.8	0.6
Thrombocytes	180-320	250	270	250	250	250
Leukocytes, g/l	4-8.8	4.4	6.2	6.0	4.8	5.0
Eosinophils	≤6 %	4	5	3	2	3
Basophils	≤1 %	0.5	1	1	1	1
Stab neutrophils	≤6 %	5	4	6	4	3
Segmenticulars	≤70 %	62.5	52	51	57	63
Lymphocytes	20-40 %	24	32	34	32	28
Monocytes	2-9 %	4	6	5	4	2
Anisocytosis	n/a	+	+	+	+	+
Poikilocytosis	n/a	+	+	+	+	+
ESR	1-10	22	5	8	10	8

Table 5.2

Immunology

Parameters	Norm	Prior to CT	After CT (day)	
			14th	60th
Lymphocytes abs. in 1 μl	720-3600	1056	2040	1536
T-lymphocytes (CD_3)	701-2005	640	1420	1100
CD_3, %	55-83	61	69.6	71.6
T-helpers (CD_4)	357-1254	215	817	519
CD_4, %	27-56	20.4	40.0	33.8
T-suppressors (CD_8)	146-810	120	318	463
CD_8, %	15-37	11.4	15.6	30.1
CD_4/CD_8 ratio	0.6-2.8	1.8	2.6	1.12
NK-cells, $CD_{16}+CD_{56}$	70-350	112	211	347
$CD_{16}+CD_{56}$, %	4-18	10.6	10.3	22.6
B-lymphocytes (CD_{19})	22-530	207	542	483
CD_{19}, %	4-24	19.6	26.6	31.4
B-2 microglobulin	0.8-2.6	0.94	2.8	2.4
Immunoglobulins:				
A, g/l	1.03-4.04	0.80	3.2	3.3
G, g/l	6.64-14.0	17.3	16.2	9.5
M, g/l	0.55-1.41	0.4	0.7	0.6

Table 6.1

Peripheral Blood Count

Parameters	Norm	03.29.1997	07.08.1997	07.14.1997	07.22.1997	12.02.1997
Erythrocytes, T/l	3.7-4.7	3.8	2.8	4.1	4.0	4.5
Hemoglobin, g/l	115-145	120	95	128	130	125
Color index	0.82-1.05	0.9	0.8	0.9	0.9	0.9
Reticulocytes, %	0.2-1.2	0.8	0.8	1.2	0.8	0.6
F-hemoglobin, %	n/a			1.4	0.8	1.2
Thrombocytes	180-320	250	270	300	250	270
Leukocytes, g/l	4-8.8	5.4	6.2	6.0	5.8	4.6
Eosinophils	≤6 %	4	3	4	2	1
Basophils	≤1 %	1	1	1	0	1
Myelocytes	n/a	-	-	-	-	-
Metamyelocytes	n/a	-	-	1	-	-
Stab neutrophils	≤6 %	6	5	7	3	4
Segmenticulars	≤70 %	56	64	48	60	60
Lymphocytes	20-40 %	27	23	34	30	32
Monocytes	2-9 %	6	4	5	5	2
Anisocytosis	n/a	-	+	+	+	+
Poikilocytosis	n/a	-	+	+	+	+
ESR	2-15	12	25	5	8	12

Table 6.2

Immunology

Parameters	Standard	03.29.1997	07.08.1997	07.22.1997	12.02.1997
Lymphocytes abs. in 1 µl	720-3600	1350	1420	1740	1472
T-lymphocytes (CD_3)	701-2005	1035	656	1235	927
CD_3, %	55-83	77	46	71	63
T-helpers (CD_4)	357-1254	740	314	713	765
CD_4, %	27-56	55	22	41	52
T-suppressors (CD_8)	146-810	347	442	487	500
CD_8, %	15-37	26	31	28	34
CD_4/CD_8 ratio	0.6-2.8	2.1	0.7	1.5	1.5
NK-cells, CD_{16}+CD_{56}	70-350	260	214	278	250
CD_{16}+CD_{56}, %	4-18	19	15	16	17
B-lymphocytes (CD_{19})	22-530	209	628	539	412
CD_{19}, %	4-24	15	44	31	28
B-2 microglobulin	0.8-2.6	1.4	2.4	2.3	2.4
Immunoglobulins:					
A, g/l	0.54-3.43	2.5	2.3	3.2	3.1
G, g/l	5.87-16.3	14.7	21.4	18.0	16.3
M, g/l	0.37-1.95	0.8	1.6	1.2	1.1

Table 7.1

Peripheral Blood Count

Parameters	Norm	11.12.1995	01.19.1996	02.28.1996	10.16.1996
Erythrocytes, T/l	3.7-4.7	3.2	3.0	4.2	4.4
Hemoglobin, g/l	115-145	97	90	123	125
Color index	0.82-1.05	0.8	0.8	0.9	0.9
Reticulocytes, %	0.2-1.2	0.6	0.8	1.1	0.6
Thrombocytes	180-320	250	170	320	300
Leukocytes, g/l	4-8.8	3.2	4.2	5.1	4.5
Eosinophils	≤6 %	4	3	2	4
Basophils	≤1 %	1	1	1	1
Stab neutrophils	≤6 %	4	5	6	4
Segmenticulars	≤70 %	67	68	54	56
Lymphocytes	20-40 %	18	15	35	32
Monocytes	2-9 %	6	8	2	4
Anisocytosis	n/a	+	+	+	+
Poikilocytosis	n/a	+	+	+	
ESR	2-15	24	32	12	8

Table 7.2

Immunology

Parameters	Norm	11.12. 1995	01.19. 1996	02.28. 1996	10.16. 1996
Lymphocytes abs. in 1 µl	720-3600	576	630	1785	1440
T-lymphocytes (CD_3)	701-2005	179	170	857	734
CD_3, %	55-83	31	27	48	51
T-helpers (CD_4)	357-1254	138	158	607	605
CD_4, %	27-56	24	25	34	42
T-suppressors (CD_8)	146-810	69	145	482	360
CD_8, %	15-37	12	23	27	25
CD_4/CD_8 ratio	0.6-2.8	2.0	1.09	1.25	0.68
NK-cells, $CD_{16}+CD_{56}$	70-350	17	32	214	158
$CD_{16}+CD_{56}$, %	4-18	3	5	12	11
B-lymphocytes (CD_{19})	22-530	92	132	179	187
CD_{19}, %	4-24	16	21	14	13
B-2 microglobulin	0.8-2.6	2.8	3.1	2.2	1.8
Immunoglobulins: A, g/l	0.54-3.43	0.4	0.7	1.2	1.4
G, g/l	5.87-16.3	14.3	16.4	8.2	5.9
M, g/l	0.37-1.95	0.6	2.2	1.8	1.2

Table 8.1

Peripheral Blood Count

Parameters	Norm	03.03.1994	03.08.1994	03.14.1994	03.28.1994
Erythrocytes, T/l	4.0-5.5	2.4	2.2	3.8	4.5
Hemoglobin, g/l	132-165	76	70	132	142
Color index	0.82-1.05	0.8	0.8	0.9	0.9
Reticulocytes, %	0.2-1.2	0.6	1.2	1.6	0.8
F-hemoglobin, %	-	-	0.6	1.2	1.1
Thrombocytes	180-320	250	270	280	250
Leukocytes, g/l	4-8.8	3.8	5.2	5.6	4.6
Eosinophils	≤6 %	3	2	2	3
Basophils	≤1 %	1	1	1	1
Myelocytes	n/a	-	1	-	-
Metamyelocytes	n/a	-	2	2	-
Stab neutrophils	≤6 %	4	6	6	5
Segmenticulars	≤70 %	59	59	48	55
Lymphocytes	20-40 %	28	25	36	30
Monocytes	2-9 %	5	4	5	6
ESR	1-10	44	16	22	28

Table 8.1 (continued)

Peripheral Blood Count

Parameters	Norm	05.09. 1994	05.12. 1994	05.19. 1994	07.21. 1994	12.17. 1994
Erythrocytes, T/l	4.0-5.5	3.2	3.4	4.6	4.4	4.3
Hemoglobin, g/l	132-165	90	100	138	140	135
Color index	0.82-1.05	0.9	0.9	0.9	0.9	0.9
Reticulocytes, %	0.2-1.2	0.3	1.2	0.6	0.6	-
F-hemoglobin, %	n/a	-	0.8	1.2	0.6	0.8
Thrombocytes	180-320	170	320	300	280	270
Leukocytes, g/l	4-8.8	0.8	3.2	4.8	5.2	4.6
Eosinophils	≤6 %	-	2	1	3	4
Basophils	≤1 %	1	1	1	1	-
Myelocytes	n/a	-	1	-	-	-
Metamyelocytes	n/a	-	2	-	-	-
Stab neutrophils	≤6 %	3	8	6	4	5
Segmenticulars	≤70 %	52	55	58	54	62
Lymphocytes	20-40 %	42	27	30	32	22
Monocytes	2-9 %	2	4	4	6	7
ESR	1-10	55	30	26	16	15

Table 8.1 (continued)

Peripheral Blood Count

Parameters	Norm	12.28. 1994	02.03. 1995	07.06. 1996	03.11. 1997	04.16. 1997	09.12. 1997
Erythrocytes, T/l	4.0-5.5	5.0	4.3	4.8	4.2	4.5	4.2
Hemoglobin, g/l	132-165	145	137	142	130	136	132
Color index	0.82-1.05	0.9	0.9	0.9	0.9	0.9	0.9
Reticulocytes, %	0.2-1.2	-	0.6	-	0.7	0.9	0.6
F-hemoglobin, %	n/a	1.6	0.8	1.4	0.4	1.4	1.2
Thrombocytes	180-320	320	300	280	340	280	300
Leukocytes, g/l	4-8.8	5.6	4.4	4.7	4.1	4.7	4.5
Eosinophils	≤6 %	2	3	5	4	3	4
Basophils	≤1 %	1	1	-	1	1	1
Myelocytes	n/a	-	-	-	-	-	-
Metamyelocytes	n/a	-	-	-	-	-	-
Stab neutrophils	≤6 %	4	5	3	4	3	4
Segmenticulars	≤70 %	50	53	56	64	53	56
Lymphocytes	20-40 %	38	32	28	24	34	30
Monocytes	2-9 %	5	6	8	3	6	5
ESR	1-10	6	12	8	9	8	8

CLAIMS

1. A method for treatment of patients using embryonic cell suspensions comprising:

preparing of a suspension containing living embryonic cells selected from the group consisting of hematopoietic liver cells, hematopoietic spleen cells, combination thereof, and pharmaceutically acceptable liquid medium, 1 ml of said suspension containing:

a) nucleated cells: $5\text{-}200*10^6$,

b) colony-forming units of granulocyte/macrophage (CFU-GM) : $20\text{-}200*10^3$,

c) colony-forming units of granulocyte, erythrocyte, monocyte/macrophage, and megakaryocyte (CFU GEMM) $0.5\text{-}10*10^3$, and

d) progenitor cells, CD_{34} (PC CD_{34}) $1\text{-}20*10^6$,

and at least one administering of such suspension, prepared *ex tempore* or frozen at cryogenic temperatures and subsequently thawed, to the body of a patient,

wherein in addition to the above main suspension of embryonic tissues, at least one additional suspension is prepared, said additional suspension containing cells selected from the group consisting of hematopoietic liver stem cells, hematopoietic spleen stem cells, hepatocytes, thymocytes, epitheliocytes of the primary alimentary canal, brain nervous cells, and combination of cells of at least two said kinds, and at least one such additional suspension is administered, along with the main one, to the patient's body.

2. The method of Claim 1, **wherein** at least one said additional suspension is administered to the patient's body concurrently with the main suspension.

3. The method of Claim 2, **wherein** prior to administration to the patient's body, at least one said additional suspension is combined with the main suspension.

4. The method of Claim 1, **wherein** said main suspension and at least one said additional suspension are administered consecutively to the patient's body.

5. The method of Claim 4, **wherein** said additional suspension is administered to the patient's body after administering the main suspension.

6. The method of Claim 4, **wherein** said main suspension is administered to the patient's body after administering the additional suspension.

7. The method of Claim 1, **wherein said** main and additional suspensions are prepared from tissues of the same embryo.

ABSTRACT

A method for treatment of patients using embryonic cell suspensions comprises preparation of the main suspension containing human embryonic cells selected from the group comprising hematopoietic liver cells, hematopoietic spleen cells, combination thereof, and pharmaceutically acceptable liquid medium, and at least one additional suspension comprising cells selected from the group consisting of hematopoietic liver stem cells, hematopoietic spleen stem cells, hepatocytes, thymocytes, epitheliocytes of the primary alimentary canal, brain nervous cells of brain, and combinations of cells of at least two said kinds, and joint use of both suspensions for treatment of such internal diseases in human patients where other modern methods and means have proven to be ineffective.

6 Claims; 8 Examples; 15 Tables.

UNITED STATES

UTILITY PATENT APPLICATION DECLARATION AND POWER OF ATTORNEY – ORIGINAL APPLICATION	ATTORNEY'S DOCKET NO. 205,209

As a below named inventor, I hereby declare that:
My residence, post office address and citizenship are as stated below next to my name:
I verily believe I am the original, first and sole inventor (if only one name is listed below) or a joint inventor (if plural inventors are named below) of the invention entitled

(1) TITLE OF INVENTION: (1) METHOD FOR TREATMENT OF PATIENTS USING EMBRYONIC CELL SUSPENSIONS

the specification of which

(2) CHECK APPROPRIATE BOX:
(2) ☐ is attached hereto.
☒ was filed on December 16, 1998 as Application No. PCT/UA98/000020
and was amended on _____ (if applicable).

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.
I acknowledge my duty to disclose information of which I am aware which is material to the patentability of this application under 37 CFR 1.56(a): the invention has not been patented or made the subject of an inventor's certificate issued before the date of this application in any country foreign to the United States of America on an application filed by me or my legal representatives or assigns more than twelve months prior to this application; and as to applications for patents or inventor's certificate on the invention filed in any country foreign to the United States prior to this application by me or my legal representatives or assigns.

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EARLIEST FOREIGN APPLICATION(S), IF ANY, FILED WITHIN 12 MONTHS PRIOR TO THIS APPLICATION				
Country	Application Number	Date of Filing (day, month, year)	Date of Issue (day, month, year)	Priority Claimed Under 35 USC 119
(4)				☐ Yes ☐ No
				☐ Yes ☐ No
				☐ Yes ☐ No
ALL FOREIGN APPLICATIONS, IF ANY, FILED MORE THAN 12 MONTHS PRIOR TO THIS APPLICATION				
(4)				

I hereby claim the benefit under Title 35, United States Code § 120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, § 112. I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, § 1.56(a) which occurred between the filing date of the prior application and the national or PCT international filing date of this application.

(5) COMPLETE DATA INDICATED IF APPLICABLE
(5) _____ (Application Serial No.) (Filing date) (Status: patented, pending, abandoned)
(5) _____ (Application Serial No.) (Filing date) (Status: patented, pending, abandoned)

Power of Attorney: As a named inventor, I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and transact all business in the Patent and Trademark Office connected therewith.

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Jeffrey A. Schwab, Registration Number 24,490
Thomas E. Spath, Registration Number 25,928
Jay S. Cinamon, Registration Number 24,136
Howard R. Jaeger, Registration Number 31,376
Joseph J. Catanzaro, Registration Number 25,837
Abigail F. Cousins, Registration Number 29,292
Anthony Coppola, Registration Number 41,493

Send correspondence to:
ABELMAN, FRAYNE & SCHWAB
150 East 42nd Street
New York, New York 10017-5612

Direct telephone calls to:
Jeffrey A. Schwab, Thomas E. Spath,
Jay S. Cinamon, Howard R. Jaeger,
Joseph J. Catanzaro, Abigail F. Cousins
or Anthony Coppola at (212) 949-9022

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment or both under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

(6) DETAILS REQUIRED FOR EACH INVENTOR

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Full Name of Sole or First Inventor	Inventor's Signature	Date
Alexandr Ivanovich SMIKODUB		15/06/2001
Residence		**Citizenship**
11/12, PER.Ivana Marjanenko, app. 26, Kyiv, 03021 Ukraine UAX		UKRAINIAN
Post Office Address		
Same as above		
Full Name of Second Joint Inventor, If Any	**Inventor's Signature**	**Date**
Residence		**Citizenship**
Post Office Address		
Full Name of Third Inventor, If Any	**Inventor's Signature**	**Date**
Residence		**Citizenship**
Post Office Address		
Full Name of Fourth Joint Inventor, If Any	**Inventor's Signature**	**Date**
Residence		**Citizenship**
Post Office Address		
Full Name of Fifth Joint Inventor, If Any	**Inventor's Signature**	**Date**
Residence		**Citizenship**
Post Office Address		
Full Name of Sixth Joint Inventor, If Any	**Inventor's Signature**	**Date**
Residence		**Citizenship**
Post Office Address		
Full Name of Seventh Joint Inventor, If Any	**Inventor's Signature**	**Date**
Residence		**Citizenship**
Post Office Address		